in 70% ethanol for 12 hours, and embedded in paraffin. After a 30 minute treatment with 0.1% pepsin in 0.1 M HCl at room temperature to denature DNA, staining was performed using mouse monoclonal anti-BrdU antibodies (Amersham). It appeared that the VEGF-C-receptor interaction in the transgenic mice transduced a mitogenic signal, because, in contrast to littermate controls, the lymphatic endothelium of the skin from young K14-VEGF-C mice showed increased DNA synthesis as demonstrated by BrdU incorporation followed by staining with anti-BrdU antibodies. This data further confirms that VEGF-C acts as a true growth factor in mammalian tissues.

In related experiments, a similar VEGF transgene did not induce lymphatic proliferation, but caused enhanced density of hyperpermeable, tortuous blood microvessels instead.

Angiogenesis is a multistep process which includes endothelial proliferation, sprouting, and migration. See Folkman *et al.*, *J. Biol. Chem.*, *267*: 10931-10934 (1992). To estimate the contribution of such processes to the transgenic phenotype, the morphology and function of the lymphatic vessels was analysed using fluorescent microlymphography using techniques known in the art. See Leu *et al.*, *Am. J. Physiol.*, *267*: 1507-1513 (1994); and Swartz *et al.*, *Am. J. Physiol.*, *270*: 324-329 (1996). Briefly, eight-week old mice were anesthetized and placed on a heating pad to maintain a 37°C temperature. A 30-gauge needle, connected to a catheter filled with a solution of FITC-dextran 2M (8 mg/ml in PBS), was injected intradermally into the tip of the tail. The solution was infused with a constant pressure of 50 cm water (averaging roughly 0.01 microliters per minute flow rate) until the extent of network filling remained constant (approximately 2 hours). Flow rate and fluorescence intensity were monitorerd continuously throughout the experiment. In these experiments, a typical honeycomb-like network with similar mesh sizes was observed in both control and transgenic mice, but the diameter of lymphatic vessels was about twice as large in the transgenic mice, as summarized in the table below. (The intravital fluorescence microscopy of blood vessels

was performed as has been described in the art. See Fukumura *et al.*, *Cancer Res.*, *55*: 4824-4829 (1995).)

Structural parameters of lymphatic and blood vessel networks				
		transgenic	**control**	**P-value****
lymphatic vessels*		(n=4)	(n=5)	
	diameter	**142.3±26.2**	**68.2±21.7**	**.0143**
	horizontal mesh size***	1003±87.1	960.8±93.1	.2207
	Vertical mesh size	507.3±58.9	488.8±59.9	.5403
		(n=3)	(n=6)	
blood vessels	median diameter	8.3±0.6	7.6±1.1	.1213
	vessel density, cm/cm^2	199.2±6.6	216.4±20.0	.3017

n=number of aminals
* mean (μm)±SD
**Mann-Whitney test
***mesh size describes vessel density

Some dysfunction of the abnormal vessels was indicated by the fact that it took longer for the dextran to completely fill the abnormal vessels. Injection of FITC-dextran into the tail vein, followed by fluorescence microscopy of the ear, showed that the blood vascular morphology was unaltered and leukocyte rolling and adherence appeared normal in the transgenic mice. These results suggest that the endothelial proliferation induced by VEGF-C leads to hyperplasia of the superficial lymphatic network but does not induce the sprouting of new vessels.

These effects of VEGF-C overexpression are unexpectedly specific, especially since, as described in other examples, VEGF-C is also capable of binding to and activating VEGFR-2, which is the major mitogenic receptor of blood vessel endothelial cells. In culture, high concentrations of VEGF-C stimulate the growth and migration of bovine capillary endothelial cells which express VEGFR-2, but not significant amounts of VEGFR-3. In addition, VEGF-C induces vascular permeability in the Miles assay [Miles,

A. A., and Miles, E. M., *J. Physiol.*, *118*:228-257 (1952); and Udaka, *et al.*, *Proc. Soc. Exp. Biol. Med.*, *133*:1384-1387 (1970)], presumably via its effect on VEGFR-2. VEGF-C is less potent than VEGF in the Miles assay, 4- to 5-fold higher concentrations of VEGF-C ΔNΔCHis being required to induce the same degree of permeability. *In vivo*, the specific effects of VEGF-C on lymphatic endothelial cells may reflect a requirement for the formation of VEGFR-3xVEGFR-2 heterodimers for endothelial cell proliferation at physiological concentrations of the growth factor. Such possible heterodimers may help to explain how three homologous VEGFs exert partially redundant, yet strikingly specific biological effects.

The foregoing *in vivo* data indicates utilities for both (i) VEGF-C polypeptides and polypeptide variants and analogs having VEGF-C biological activity, and (ii) anti-VEGF-C antibodies and VEGF-C antagonists that inhibit VEGF-C activity (*e.g.*, by binding VEGF-C or interfering with VEGF-C/receptor interactions. For example, the data indicates a therapeutic utility for VEGF-C polypeptides in patients wherein growth of lymphatic tissue may be desirable (e.g., in patients following breast cancer or other surgery where lymphatic tissue has been removed and where lymphatic drainage has therefore been compromised, resulting in swelling; or in patients suffering from elephantiasis). The data indicates a therapeutic utility for anti-VEGF-C antibody substances and VEGF-C antagonists for conditions wherein growth-inhibition of lymphatic tissue may be desirable (e.g., treatment of lymphangiomas). Accordingly, methods of administering VEGF-C and VEGF-C variants, analogs, and antagonists are contemplated as methods and materials of the invention.

Example 30

Expression of VEGF-C and Flt4 in the Developing Mouse

Embryos from a 16-day post-coitus pregnant mouse were prepared and fixed in 4% paraformaldehyde (PFA), embedded in paraffin, and sectioned at 6 μm. The sections were placed on silanated microscope slides and treated with xylene, rehydrated, fixed for 20 minutes in 4% PFA, treated with proteinase K (7mg/ml; Merck, Darmstadt, Germany) for 5 minutes at room temperature, again fixed in 4% PFA and treated with acetic anhydride, dehydrated in solutions with increasing ethanol concentrations, dried and used for *in situ* hybridization.

In situ hybridization of sections was performed as described (Västrik *et al.*, *J. Cell Biol.*, *128:*1197-1208 (1995)). A mouse VEGF-C antisense RNA probe was generated from linearized pBluescript II SK+ plasmid (Stratagene Inc.), containing a fragment corresponding to nucleotides 499-979 of mouse VEGF-C cDNA, where the noncoding region and the BR3P repeat were removed by Exonuclease III treatment. The fragment had been cloned into the *Eco*RI and *Hin*dIII sites of pBluescript II SK+. Radiolabeled RNA was synthesized using T7 RNA Polymerase and [^{35}S]-UTP (Amersham, Little Chalfont, UK). About two million cpm of the VEGF-C probe was applied per slide. After an overnight hybridization, the slides were washed first in 2x SSC and 20-30 mM DDT for 1 hour at 50°C. Treatment continued with a high stringency wash, 4x SSC and 20 mM DTT and 50% deionized formamide for 30 minutes at 65°C followed by RNase A treatment (20 µg/ml) for 30 minutes at 37°C. The high stringency wash was repeated for 45 minutes. Finally, the slides were dehydrated and dried for 30 minutes at room temperature. The slides were dipped into photography emulsion and exposed for 4 weeks. Slides were developed using Kodak D-16 developer, counterstained with hematoxylin and mounted with Permount (FisherChemical).

For *in situ* hybridizations of Flt4 sequences, a mouse Flt4 cDNA fragment covering bp 1-192 of the published sequence (Finnerty *et al.*, *Oncogene, 8:*2293-2298 (1993)) was used, and the above-described protocol was followed, with the following exceptions. Approximately one million cpm of the Flt4 probe were applied to each slide. The stringent washes following hybridization were performed in 1x SSC and 30 mM DTT for 105 minutes.

Darkfield and lightfield photomicrographs from these experiments are presented in commonly-owned PCT patent application PCT/FI96/00427, filed August 01, 1996, incorporated by reference herein. Observations from the photomicrographs are summarized below.

The most prominently Flt4-hybridizing structures appeared to correspond to the developing lymphatic and venous endothelium. A plexus-like endothelial vascular structure surrounding the developing nasopharyngeal mucous membrane was observed. The most prominent signal using the VEGF-C probe was obtained from the posterior part of the developing nasal conchae, which in higher magnification showed the epithelium surrounding loose connective tissue/forming cartilage. This structure gave a strong *in situ*

hybridization signal for VEGF-C. With the VEGF-C probe, more weakly hybridizing areas were observed around the snout, although this signal is much more homogeneous in appearance. Thus, the expression of VEGF-C is strikingly high in the developing nasal conchae.

The conchae are surrounded with a rich vascular plexus, important in nasal physiology as a source for the mucus produced by the epithelial cells and for warming inhaled air. It is suggested that VEGF-C is important in the formation of the concheal venous plexus at the mucous membranes, and that it may also regulate the permeability of the vessels needed for the secretion of nasal mucus. Possibly, VEGF-C and its derivatives, and antagonists, could be used in the regulation of the turgor of the conchal tissue and mucous membranes and therefore the diameter of the upper respiratory tract, as well as the quantity and quality of mucus produced. These factors are of great clinical significance in inflammatory (including allergic) and infectious diseases of the upper respiratory tract. Accordingly, the invention contemplates the use of the materials of the invention, including VEGF-C, Flt4, and their derivatives, in methods of diagnosing and treating inflammatory and infectious conditions affecting the upper respiratory tract, including nasal structures.

Example 31

Characterization of the exon-intron organization of the human VEGF-C gene

Two genomic DNA clones covering exons 1, 2, and 3 of the human VEGF-C gene were isolated from a human genomic DNA library using VEGF-C cDNA fragments as probes. In particular, a human genomic library in bacteriophage EMBL-3 lambda (Clontech) was screened using a PCR-generated fragment corresponding to nucleotides 629-746 of the human VEGF-C cDNA (SEQ ID NO: 7). One positive clone, designated "lambda 3," was identified, and the insert was subcloned as a 14 kb *Xho*I fragment into the pBluescript II (pBSK II) vector (Stratagene). The genomic library also was screened with a labeled 130 bp *Not*I-*Sac*I fragment from the 5'-noncoding region of the VEGF-C cDNA (the *Not*I site is in the polylinker of the cloning vector; the *Sac*I site corresponds to nucleotides 92-97 of SEQ ID NO: 7). Two positive clones, designated "lambda 5" and "lambda 8," were obtained. Restriction mapping analysis showed that

clone lambda 3 contains exons 2 and 3, while clone lambda 5 contains exon 1 and the putative promoter region.

Three genomic fragments containing exons 4, 5, 6 and 7 were subcloned from a genomic VEGF-C P1 plasmid clone. In particular, purified DNA from a genomic P1 plasmid clone 7660 (Paavonen *et al.*, *Circulation, 93*: 1079-1082 (1996)) was used. *Eco*RI fragments of the P1 insert DNA were ligated into pBSK II vector. Subclones of clone 7660 which contained human VEGF-C cDNA homologous sequences were identified by colony hybridization, using the full-length VEGF-C cDNA as a probe. Three different genomic fragments were identified and isolated, which contained the remaining exons 4-7.

To determine the genomic organization, the clones were mapped using restriction endonuclease cleavage. Also, the coding regions and exon-intron junctions were partially sequenced. The result of this analysis is depicted in Figures 11A and 12. The sequences of all intron-exon boundaries (Fig. 11A, SEQ ID NOs: 24-35) conformed to the consensus splicing signals (Mount, *Nucl. Acids Res.*, *10*: 459-472 (1982)). The length of the intron between exon 5 and 6 was determined directly by nucleotide sequencing and found to be 301 bp. The length of the intron between exons 2 and 3 was determined by restriction mapping and Southern hybridization and was found to be about 1.6 kb. Each of the other introns is over 10 kb in length.

A similar analysis was performed for the murine genomic VEGF-C gene. The sequences of murine VEGF-C intron-exon boundaries are depicted in Figure 11B and SEQ ID NOs: 36-47.

The restriction mapping and sequencing data indicated that the VEGF-C signal sequence and the first residues of the N-terminal propeptide are encoded by exon 1. The second exon encodes the carboxy-terminal portion of the N-terminal propeptide and the amino terminus of the VEGF homology domain. The most conserved sequences of the VEGF homology domain are distributed in exons 3 (containing 6 conserved cysteine residues) and 4 (containing 2 cys residues). The remaining exons encode cysteine-rich motifs of the type C-6X-C-10X-CRC (exons 5 and 7) and a fivefold repeated motif of type C-6X-B-3X-C-C-C, which is typical of a silk protein.

To further characterize the human VEGF-C gene promoter, the lambda 5 clone was further analyzed. Restriction mapping of this clone using a combination of

single- and double-digestions and Southern hybridizations indicated that it includes: (1) an approximately 6 kb region upstream of the putative initiator ATG codon, (2) exon 1, and (3) at least 5 kb of intron I of the VEGF-C gene.

A 3.7 kb *Xba* I fragment of clone lambda 5, containing exon 1 and 5' and 3' flanking sequences, was subcloned and further analyzed. As reported previously, a major VEGF-C mRNA band migrates at a position of about 2.4 kb. Calculating from the VEGF-C coding sequence of 1257 bp and a 391 bp 3' noncoding sequence plus a polyA sequence of about 50-200 bp, the mRNA start site was estimated to be about 550-700 bp upstream of the translation initiation codon.

RNase protection assays were employed to obtain a more precise localization of the mRNA start site. The results of these experiments indicated that the RNA start site in the human VEGF-C gene is located 539 bp upstream of the ATG translational initiation codon.

To further characterize the promoter of the human VEGF-C gene, a genomic clone encompassing about 2.4 kb upstream of the translation initiation site was isolated, and the 5' noncoding cDNA sequence and putative promoter region were sequenced. The sequence obtained is set forth in SEQ ID NO: 48. (The beginning of the VEGF-C cDNA sequence set forth in SEQ ID NO: 7 corresponds to position 2632 of SEQ ID NO: 48; the translation initiation codon corresponds to positions 2983-2985 of SEQ ID NO: 48.) Similar to what has been observed with the VEGF gene, the VEGF-C promoter is rich in G and C residues and lacks consensus TATA and CCAAT sequences. Instead, it has numerous putative binding sites (5'-GGGCGG-3' or 5'-CCGCCC-3') for Sp1, a ubiquitous nuclear protein that can initiate transcription of TATA-less genes. See Pugh and Tjian, *Genes and Dev., 5*:105-119 (1991). In addition, sequences upstream of the VEGF-C translation start site were found to contain frequent consensus binding sites for the AP-2 factor (5'-GCCN$_3$GCC-3') and binding sites for the AP-1 factor (5'-TKASTCA-3'). Binding sites for regulators of tissue-specific gene expression, like NFkB and GATA, are located in the distant part of VEGF-C promoter. This suggests that the cAMP-dependent protein kinase and protein kinase C, as activators of AP-2 transcription factor [Curran and Franza, *Cell, 55*:395-397 (1988)], mediate VEGF-C transcriptional regulation.

The VEGF-C gene is abundantly expressed in adult human tissues, such as heart, placenta, ovary and small intestine, and is induced by a variety of factors. Indeed, several potential binding sites for regulators of tissue-specific gene expression, like NFkB (5'-GGGRNTYYC-3') and GATA, are located in the distal part of the VEGF-C promoter. For example, NFkB is known to regulate the expression of tissue factor in endothelial cells. Also, transcription factors of the GATA family are thought to regulate cell-type specific gene expression.

Unlike VEGF, the VEGF-C gene does not contain a binding site for the hypoxia-inducible factor, HIF-1 (Levy *et al., J. Biol. Chem., 270:* 13333-13340 (1995)). This finding suggests that if the VEGF-C mRNA is regulated by hypoxia, the mechanism would be based mainly on the regulation of mRNA stability. In this regard, numerous studies have shown that the major control point for the hypoxic induction of the VEGF gene is the regulation of the steady-state level of mRNA. See Levy *et al., J. Biol. Chem., 271*: 2746-2753 (1996). The relative rate of VEGF mRNA stability and decay is considered to be determined by the presence of specific sequence motifs in its 3' untranslated region (UTR), which have been demonstrated to regulate mRNA stability. (Chen and Shyu, *Mol. Cell Biol., 14*: 8471-8482 (1994)). The 3'-UTR of the VEGF-C gene also contains a putative motif of this type (TTATTT), at positions 1873-1878 of SEQ ID NO: 7.

To identify DNA elements important for basal expression of VEGF-C in transfected cells, a set of luciferase reporter plasmids containing serial 5' deletions through the promoter region was constructed. Restriction fragments of genomic DNA containing 5' portions of the first exon were cloned into the polylinker of the pGL3 reporter vector (Promega) and confirmed by sequencing. About 10 μg of the individual constructs in combination with 2 μg of pSV2-ß-galactosidase plasmid (used as a control of transfection efficiency) were transfected into HeLa cells using the calcium phosphate-mediated transfection method. Two days after transfection, the cells were harvested and subjected to the luciferase assay. The luciferase activity was normalized to that of the pGL3 control vector driven by SV40 promoter/enhancer.

As depicted in Fig. 3, the 5.5 kb *Xho*I-*Rsr*II fragment of clone lambda 5 gave nearly 9-fold elevated activity when compared with a promoterless vector. Deletion of a 5' *Xho*I-*Hind*III fragment of 2 kb had no effect on the promoter activity. The activity

of the 1.16 kb *Xba*I-*Rsr*II fragment was about twice that of the pGL3 basic vector, while the activity of the same fragment in the reverse orientation was at background level. Further deletion of the XbaI-SacI fragment caused an increase in the promoter activity, suggesting the presence of silencer elements in the region from -1057 to -199 (i.e., 199 to 1057 bp upstream from the transcription initiation site). The shortest fragment (*Sac*II-*Rsr*II) yielded only background activity, which was consistent with the fact that the mRNA initiation site was not present in this construct.

To determine whether further sequences in the first exon of human VEGF-C are important for basal expression, an *Rsr*II fragment spanning nucleotides 214-495 (i.e., 214-495 bp downstream from the transcription initiation site) was subcloned in between of *Xba*I-*Rsr*II fragment and the luciferase reporter gene. Indeed, the obtained construct showed an 50 % increase in activity when compared with the *Xba*I-*Rsr*II construct.

The VEGF gene has been shown to be up-regulated by a number of stimuli including serum derived growth factors. To find out whether the VEGF-C gene also can be stimulated by serum, RNA from serum-starved and serum-stimulated HT1080 cells was subjected to primer extension analysis, which demonstrated that VEGF-C mRNA is up-regulated by serum stimulation.

Additional serum stimulation experiments indicated that the serum stimulation leads to increased VEGF-C promoter activity. Cells were transfected as described above and 24 h after transfection changed into medium containing 0.5% bovine serum albumin. Cells were then stimulated with 10 % fetal calf serum for 4 hours and analyzed. The XbaI-RsrII promoter construct derived from lambda 5 yielded a twofold increased activity upon serum stimulation, while the same fragment in the reverse orientation showed no response. All other promoter constructs also showed up-regulation, ranging from 1.4 to 1.6 fold (Fig. 3).

Example 32

Identification of a VEGF-C splice variant

As reported in Example 16, a major 2.4 kb VEGF-C mRNA and smaller amounts of a 2.0 kb mRNA are observable. To clarify the origin of these RNAs, several additional VEGF-C cDNAs were isolated and characterized. A human fibrosarcoma cDNA library from HT1080 cells in the lambda gt11 vector (Clontech, product

#HL1048b) was screened using a 153 bp human VEGF-C cDNA fragment as a probe as described in Example 10. See also Joukov *et al., EMBO J., 15:*290-298 (1996). Nine positive clones were picked and analyzed by PCR amplification using oligonucleotides 5'-CACGGCTTATGCAAGCAAAG-3' (SEQ ID NO: 49) and 5'-AACACAGTTTTCCATAATAG-3' (SEQ ID NO: 50) These oligonucleotides were selected to amplify the portion of the VEGF-C cDNA corresponding to nucleotides 495-1661 of SEQ ID NO: 7. PCR was performed using an annealing temperature of 55°C and 25 cycles.

The resultant PCR products were electrophoresed on agarose gels. Five clones out of the nine analyzed generated PCR fragments of the expected length of 1147 base pairs, whereas one was slightly shorter. The shorter fragment and one of the fragments of expected length were cloned into the pCRTMII vector (Invitrogen) and analyzed by sequencing. The sequence revealed that the shorter PCR fragment had a deletion of 153 base pairs, corresponding to nucleotides 904 to 1055 of SEQ ID NO: 7. These deleted bases correspond to exon 4 of the human and mouse VEGF-C genes, schematically depicted in Figs. 13A and 13B. Deletion of exon 4 results in a frameshift, which in turn results in a C-terminal truncation of the full-length VEGF-C precursor, with fifteen amino acid residues translated from exon 5 in a different frame than the frame used to express the full-length protein. Thus, the C-terminal amino acid sequence of the resulting truncated polypeptide would be --Leu (181)-Ser-Lys-Thr-Val-Ser-Gly-Ser-Glu-Gln-Asp-Leu-Pro-His-Glu-Leu-His-Val-Glu(199) (SEQ ID NO: 51). The polypeptide encoded by this splice variant would not contain the C-terminal cleavage site of the VEGF-C precursor. Thus, a putative alternatively spliced RNA form lacking conserved exon 4 was identified in HT-1080 fibrosarcoma cells and this form is predicted to encode a protein of 199 amino acid residues, which could be an antagonist of VEGF-C.

Example 33

VEGF-C is similarly processed in different cell cultures in vitro

To study whether VEGF-C is similarly processed in different cell types, 293 EBNA cells, COS-1 cells and HT-1080 cells were transfected with wild type human VEGF-C cDNA and labelled with Pro-Mix™ as described in Example 22. The conditioned media from the cultures were collected and subjected to immunoprecipitation using

antiserum 882 (described in Example 21, recognizing a peptide corresponding to amino acids 104-120 of SEQ ID NO: 8). The immunoprecipitated polypeptides were separated via SDS-PAGE, and detected via autoradiography. The major form of secreted recombinant VEGF-C observed from all cell lines tested is a 29/32 kD doublet. These two polypeptides are bound to each other by disulfide bonds, as described in Example 22. A less prominent band of approximately 21 kD also was detected in the culture media. Additionally, a non-processed VEGF-C precursor of 63 kDa was observed. This form was more prominent in the COS-1 cells, suggesting that proteolytic processing of VEGF-C in COS cells is less efficient than in 293 EBNA cells. Endogenous VEGF-C (in non-transfected cells) was not detectable under these experimental conditions in the HT-1080 cells, but was readily detected in the conditioned medium of the PC-3 cells. Analysis of the subunit polypeptide sizes and ratios in PC-3 cells and 293 EBNA cells revealed strikingly similar results: the most prominent form was a doublet of 29/32 kDa and a less prominent form the 21 kD polypeptide. The 21 kD form produced by 293 EBNA cells was not recognized by the 882 antibody in the Western blot, although it is recognized when the same antibody is used for immunoprecipitation (see data in previous examples). As reported in Example 21, cleavage of the 32 kD form in 293 EBNA cells occurs between amino acid residues 111 and 112 (SEQ ID NO: 8), downstream of the cleavage site in PC-3 cells (between residues 102 and 103). Therefore, the 21 kD form produced in 293 EBNA cells does not contain the complete N-terminal peptide used to generate antiserum 882. In a related experiment, PC-3 cells were cultured in serum-free medium for varying periods of time (1 - 8 days) prior to isolation of the conditioned medium. The conditioned medium was concentrated using a Centricon device (Amicon, Beverly, USA) and subjected to Western blotting analysis using antiserum 882. After one day of culturing, a prominent 32 kD band was detected. Increasing amounts of a 21-23 kD form were detected in the conditioned media from 4 day and 8 day cultures. The diffuse nature of this polypeptide band, which is simply called the 23 kD polypeptide in example 5 and several subsequent examples, is most likely due to a heterogenous and variable amount of glycosylation. These results indicate that, initially, the cells secrete a 32 kD polypeptide, which is further processed or cleaved in the medium to yield the 21-23 kD form. The microheterogeneity of this polypeptide band would then arise from the variable glycosylation degree and, from microheterogeneity of the processing cleavage sites, such

as obtained for the amino terminus in PC-3 and 293 EBNA cell cultures. The carboxyl terminal cleavage site could also vary, examples of possible cleavage sites would be between residues 225-226, 226-227 and 227-228 as well as between residues 216-217. Taken together, these data suggest the possibility that secreted cellular protease(s) are responsible for the generation of the 21-23 kD form of VEGF-C from the 32 kD polypeptide. Such proteases could be used in vitro to cleave VEGF-C precursor proteins in solution during the production of VEGF-C, or used in cell culture and in vivo to release biologically active VEGF-C.

Example 34

Differential binding of VEGF-C forms by the extracellular domains of VEGFR-3 and VEGFR-2

In two parallel experiments, 293 EBNA cells were transfected with a construct encoding recombinant wild type VEGF-C or a construct encoding VEGF-C ΔNΔCHis (Example 28) and about 48 hours after transfection, metabolically labelled with Pro-Mix™ as described in previous examples. The media were collected from mock-transfected and transfected cells and used for receptor binding analyses.

Receptor binding was carried out in binding buffer (PBS, 0.5% BSA, 0.02% Tween 20, 1 microgram/ml heparin) containing approximately 0.2 microgram of either (a) a fusion protein comprising a VEGFR-3 extracellular domain fused to an immunoglobulin sequence (VEGFR-3-Ig) or (b) a fusion protein comprising VEGFR-2 extracellular domain fused to an alkaline phosphatase sequence (VEGFR-2-AP; Cao *et al.*, *J. Biol. Chem. 271*:3154-62 (1996)). As a control, similar aliquots of the 293 EBNA conditioned media were mixed with 2 µl of anti-VEGF-C antiserum (VEGF-C IP).

After incubation for 2 hours at room temperature, anti-VEGF-C antibodies and VEGFR-3-Ig protein were adsorbed to protein A-sepharose (PAS) and VEGFR-2-AP was immunoprecipitated using anti-AP monoclonal antibodies (Medix Biotech, Genzyme Diagnostics, San Carlos, CA, USA) and protein G-sepharose. Complexes containing VEGF-C bound to VEGFR-3-Ig or VEGFR-2-AP were washed three times in binding buffer, twice in 20 mM Tris-HCl (pH 7.4) and VEGF-C immunoprecipitates were washed three times in RIPA buffer and twice in 20 mM tris-HCl (pH 7.4) and analyzed via SDS-PAGE under reducing and nonreducing conditions. As a control, the same media were

precipitated with antiAP and protein G-sepharose (PGS) or with PAS to control for possible nonspecific adsorption.

These experiments revealed that VEGFR-3 bound to both the 32/29 kD and 21-23 kD forms of recombinant VEGF-C, whereas VEGFR-2 bound preferentially to the 21-23 kD component from the conditioned media. In addition, small amounts of 63 kD and 52 kD VEGF-C forms were observed binding with VEGFR-3. Further analysis under nonreducing conditions indicates that a great proportion of the 21-23 kD VEGF-C bound to either receptor does not contain interchain disulfide bonds. These findings reinforce the results that VEGF-C binds VEGFR-2. This data suggests a utility for recombinant forms of VEGF-C which are active towards VEGFR-3 only or which are active towards both VEGFR-3 and VEGFR-2. On the other hand, these results, together with the results in Example 28, do not eliminate the possibility that the 32/29 kD dimer binds VEGFR-3 but does not activate it. The failure of the 32/29 kD dimer to activate VEGFR-3 could explain the finding that conditioned medium from the N-His VEGF-C transfected cells induced a less prominent tyrosine phosphorylation of VEGFR-3 than medium from VEGF-C ΔNΔCHis transfected cells, even though expression of the former polypeptide was much higher. Stable VEGF-C polypeptide mutants that bind to a VEGF-C receptor but fail to activate the receptor are useful as VEGF-C antagonists.

Example 35

Discovery of VEGF-C analogs that selectively bind to and activate VEGFR-3, but not VEGFR-2

To further identify the cysteine residues of VEGF-C that are critical for retaining VEGF-C biological activities, an additional VEGF-C mutant, designated VEGF-CΔNΔCHisC156S, was synthesized, in which the cysteine residue at position 156 of the 419 amino acid VEGF-C precursor (SEQ ID NO: 8; Genbank accession number X94216) was replaced with a serine residue.

The mutagenesis procedure was carried out using the construct of VEGF-CΔNΔCHis (see Example 28), cloned in the pALTER vector, and the *Altered sites II in vitro mutagenesis system* of Promega. An oligonucleotide 5'-GACGGACACAGATGGAGGTTTAAAG-3' (SEQ ID NO: 52) was used to introduce the desired mutation in the cDNA encoding VEGF-CΔNΔCHis. The resulting mutated

VEGF-C cDNA fragment was subcloned into the *Hind*III/*Not*I sites of the pREP-7 vector (Invitrogen), and the final construct was re-sequenced to confirm the C156S mutation. The resultant clone has an open reading frame encoding amino acids 103-225 of SEQ ID NO: 8 (with a serine codon at position 156), and further encoding a 6xHis tag.

The wildtype VEGF-C cDNA and three VEGF-C mutant constructs (VEGF-C R226,227S, VEGF-C ΔNΔCHis, and VEGF-C ΔNΔCHisC156S) were used to transfect 293 EBNA cells, which were subcultured 16 hours after transfection. About 48 hours after transfection, the media were changed to DMEM/0.1% BSA, and incubation in this medium was continued for an additional 48 hours. The resultant conditioned media were concentrated 30-fold using *Centriprep-10* (Amicon), and the amount of VEGF-C in the media was analyzed by Western blotting using the anti-VEGF-C antiserum 882 for immunodetection. Different amounts of the recombinant VEGF-C ΔNΔCHis, purified from a yeast expression system, were analyzed in parallel as reference samples to measure and equalize the VEGF-C concentrations in the conditioned media. The conditioned medium from mock-transfected cells was used to dilute the VEGF-C conditioned media to achieve equal concentrations.

An aliquot of the transfected cells were metabolically labelled for 6 hours with 100 microcuries/ml of the PRO-MIX™ L-[^{35}S] *in vitro* cell labelling mix (Amersham). The conditioned media were collected, and binding of the radioactively labelled VEGF-C proteins to the extracellular domains of VEGFR-3 and VEGFR-2 was analyzed using recombinantly produced VEGFR-3EC-Ig and VEGFR-2EC-Ig constructs (containing seven and three Ig loops of the extracellular domains of the respective receptors, fused to an immunoglobulin heavy chain constant region).

All processed VEGF-C forms secreted to the culture medium bound to VEGFR-3EC domain, with preferential binding of the 21 kDa form. When present at high concentrations, the VEGF-C forms of 58 kDa and 29/31 kDa bound to some extent non-specifically to protein A Sepharose.

The VEGFR-2EC domain preferentially bound the mature 21 kDa form of wildtype VEGF-C and VEGF-CΔNΔCHis. Significantly, VEGF-CΔNΔCHisC156S failed to bind the VEGFR2-EC.

Next, the ability of the above-described VEGF-C polypeptides to compete with the ^{125}I-VEGF-CΔNΔCHis for binding to VEGFR-2 and VEGFR-3 was analyzed.

Scatchard analysis using VEGF-C ΔCΔNHis provided indications of the VEGF-C binding affinity for VEGFR-3 (K_D=135 pM) and VEGFR-2 (K_D=410 pM). Ten micrograms of the purified yeast VEGF-C ΔNΔCHis was labeled using 3 mCi of Iodine-125, carrier-free (Amersham), and an Iodo-Gen Iodination Reagent (Pierce), according to the standard protocol of Pierce. The resulting specific activity of the labeled VEGF-CΔNΔCHis was $1.25x10^5$ cpm/ng.

To study receptor binding, PAE/VEGFR-2 and PAE/VEGFR-3 cells were seeded into 24-well tissue culture plates (Nunclon), which had been coated with 2% gelatin in PBS. The ^{125}I-VEGF-C ΔNΔCHis ($2x10^5$ cpm) and different amounts of media containing equal concentrations of the non-labeled VEGF-C (wildtype and mutants) were added to each plate in Ham's F12 medium, containing 25 mM HEPES (pH 8.0), 0.1% BSA, and 0.1% NaN_3. The binding was allowed to proceed at room temperature for 90 minutes. The plates were then transferred onto ice and washed three times with ice-cold PBS containing 0.1% BSA. The cells were then lysed in 1 M NaOH, the lysates were collected, and the radioactivity was measured using a γ-counter. Binding in the presence of VEGF-C-containing conditioned medium was calculated as a percentage of binding observed in parallel control studies wherein equal volumes of medium from mock-transfected cells were used instead of VEGF-C conditioned media.

As shown in Fig. 4, left panel, all VEGF-C mutants displaced ^{125}I-VEGF-CΔNΔCHis from VEGFR-3. The efficiency of displacement was as follows: VEGF-CΔNΔCHisC156S > VEGF-CΔNΔCHis > wildtype VEGF-C > VEGF-CR226,227S. These results indicate that enhanced binding to VEGFR-3 was obtained upon "recombinant maturation" of VEGF-C. Recombinant VEGF165 failed to displace VEGF-C from VEGFR-3.

VEGF, VEGF-CΔNΔCHis, and wildtype VEGF-C all efficiently displaced labeled VEGF-CΔNΔCHis from VEGFR-2, with VEGF-CΔNΔCHis being more potent when compared to wildtype VEGF-C (Fig. 4, right panel). The non-processed VEGF-C R226,227S showed only weak competition of ^{125}I-VEGF-CΔNΔCHis.

Surprisingly, VEGF-CΔNΔCHisR156S failed to displace VEGF-CΔNΔCHis from VEGFR-2, thus confirming the above described results obtained using a soluble extracellular domain of VEGFR-2.

The ability of the above mentioned VEGF-C forms to stimulate tyrosine phosphorylation of VEGFR-3 and VEGFR-2 was also investigated. Importantly, identical dilutions of the conditioned media were used for these experiments and for the competitive binding experiments described above. A Western blot analysis of the conditioned media using anti-VEGF-C antiserum 882 was performed to confirm the approximately equal relative amounts of the factors present.

The stimulation of VEGFR-3 and VEGFR-2 autophosphorylation by the different VEGF-C forms in general correlated with their binding properties, as well as with the degree of "recombinant processing" of VEGF-C. The VEGF-CΔNΔCHisC156S appeared to be at least as potent as VEGF-CΔNΔCHis in stimulating VEGFR-3 autophosphorylation. VEGF-CΔNΔCHis showed a higher potency when compared to wildtype VEGF-C in its ability to stimulate tyrosine autophosphorylation of both VEGFR-2 and VEGFR-3. The VEGF-CR226,227S conditioned medium possessed a considerably weaker effect on autophosphorylation of VEGFR-3, and almost no effect on VEGFR-2 autophosphorylation.

Stimulation of VEGFR-2 tyrosine phosphorylation by VEGF-CΔNΔCHisC156S did not differ from that of conditioned medium from the mock transfected cells, thus confirming the lack of VEGFR-2-binding and VEGFR-2-activating properties of this mutant.

The ability of VEGF-C ΔNΔCHisC156S to alter vascular permeability *in vivo* was analyzed using the Miles assay (see Example 29). The recombinant VEGF-C forms assayed (ΔNΔCHis, ΔNΔCHisC156S) were produced by 293 cells, purified from conditioned media using Ni-NTA Superflow resin (QIAGEN) as previously described, and pretreated with 15 μg/ml of anti-human VEGF neutralizing antibody (R&D systems) to neutralize residual amounts of co-purified, endogenously produced VEGF. Eight picomoles of the various VEGF-C forms, as well as 2 pmol of recombinant human VEGF165 (R&D systems) and approximately 2 pmol of VEGF165 from the conditioned medium which were either non-treated or pretreated with the above mentioned VEGF-neutralizing antibody were injected subcutaneously to the back region of a guinea pig. The area of injection was analyzed 20 minutes after injections. Both VEGF and VEGF-C ΔNΔCHis caused increases in vascular permeability, whereas ΔNΔCHisC156S did not affect vascular permeability. The neutralizing antibody completely blocked permeability

activity of VEGF but did not affect VEGF-C activity. Residual permeability activity observed for the VEGF-containing conditioned medium even after its treatment with VEGF neutralizing antibody was presumably caused by permeability factors other than VEGF that are produced by 293 cells.

In yet another assay, the ability of VEGF-CΔNΔCHis and VEGF-CΔNΔCHisC156S to stimulate migration of bovine capillary endothelial cells in a collagen gel was analyzed. The ΔNΔCHis form dose-dependently stimulated migration, whereas the ΔNΔCHisC156S form had no significant activity in the assay.

The Miles assay also was used to assay the ability of VEGF-C R226,227S (8 pM, pretreated with anti-VEGF antibody) to induce vascular permeability. The results indicated that the ability of VEGF-C R226,227S to induce vascular permeability was much weaker when compared to wildtype and ΔNΔCHis forms of VEGF-C. Collectively, this Miles assay data is consistent with the VEGFR-2 binding and autophosphorylation data described above, and indicates that VEGF-C effect on vascular permeability is mediated via VEGFR-2.

Mitogenic signals from growth factor receptors are frequently relayed via the extracellular signal regulated kinases/mitogen activated protein kinases (ERK/MAPK) pathway into the nucleus. Purified recombinant VEGF-CΔNΔCHis and VEGF-C ΔNΔC156S produced by a Pichia expression system were used to determine MAPK pathway activation of cells expressing either VEGFR-2 or VEGFR-3. The growth factor treated cells were lysed, and activated MAPK was detected using Western blotting with antibodies against the phosphorylated forms of ERK1 and ERK2. At a concentration of 100 ng/ml, VEGF-CΔNΔCHis showed rapid activation of the ERK1 and ERK2 MAPK in both VEGFR-2- and VEGFR-3-expressing cells. In contrast, VEGF-CΔNΔC156S activated ERK1 and ERK2 exclusively in the VEGFR-3-expressing cells. At the concentrations used, both VEGF-CΔNΔCHis and VEGF-C ΔNΔC156S appeared to be equally potent in activating the MAPK through VEGFR-3. The amounts of total MAPK protein were confirmed to be similar in the treated and untreated cells, as shown by staining of the filter with p44/p42 MAPK antibodies made against a synthetic peptide of rat p42.

The foregoing data indicates that proteolytic processing of VEGF-C results in an increase in its ability to bind and to activate VEGFR-3 and VEGFR-2. Non-

processed VEGF-C is a ligand and an activator of preferentially VEGFR-3, while the mature 21/23 kDa VEGF-C form is a high affinity ligand and an activator of both VEGFR-3 and VEGFR-2.

Moreover, replacement of the cysteine residue at position 156 (of prepro-VEGF-C, SEQ ID NO: 8) creates a selective ligand and activator of VEGFR-3. This alteration inactivates the ability of processed VEGF-C to bind to VEGFR-2 and to activate VEGFR-2. Importantly, it is believed that the elimination of the cysteine at position 156 is the alteration responsible for this unexpected alteration in VEGF-C selectivity, and not the substitution of a serine per se. It is expected that replacement of the cysteine at position 156 with other amino acids, or the mere deletion of this cysteine, will also result in VEGF-C analogs having selective biological activity with respect to VEGFR-3. All such replacement and deletion analogs (collectively referred to as VEGF-C ΔC_{156} polypeptides) are contemplated as aspects of the present invention. Thus, "VEGF-C ΔC_{156} polypeptides" of the invention derived from human VEGF-C include polypeptides depicted in SEQ ID NO: 58, fragments of those polypeptides (especially fragments having an amino terminus anywhere between residues 102 and 161 of SEQ ID NO: 58 and a carboxy-terminus anywhere between residues 210 and 228 of SEQ ID NO: 58). "VEGF-C ΔC_{156} polypeptides" of the invention also include the corresponding polypeptides derived from murine, quail, and other wildtype VEGF-C polypeptides.

VEGF-C polypeptides that have the C156S mutation (or functionally equivalent mutations at position 156) and that retain biological activity with respect to VEGFR-3, such as VEGF-C ΔNΔCHisC156S, are useful in all of the same manners described above for wildtype VEGF-C proteins and biologically active fragments thereof where VEGFR-3 stimulation is desired. It is contemplated that most biologically active VEGF-C fragments and processing variants, including but not limited to the biologically active fragments and variants identified in preceding examples, will retain VEGF-C biological activity (as mediated through VEGFR-3) when a ΔC_{156} mutation is introduced. All such biologically active VEGF-C ΔC_{156} polypeptides are intended as an aspect of the present invention.

Moreover, VEGF-C forms containing the C156S mutation or equivalent mutations can be used to distinguish those effects of VEGF-C mediated via VEGFR-3 and VEGFR-2 from those obtained via only VEGFR-3. The ability of such VEGF-C

polypeptides to selectively stimulate VEGFR-3 are also expected to be useful in clinical practice, it being understood that selectivity of a pharmaceutical is highly desirable in many clinical contexts. For example, the selectivity of VEGF-C ΔC_{156} polypeptides for VEGFR-3 binding suggests a utility for these peptides to modulate VEGF-C biological activities mediated through VEGFR-3, without significant concomitant modulation of blood vessel permeability or other VEGF-C activities that are modulated through VEGFR-2.

The data presented herein also indicates a utility for ΔC_{156} polypeptides that are capable of binding VEGFR-3, but that do not retain biological activity mediated through VEGFR-3. Specifically, such forms are believed to be capable of competing with wildtype VEGF-C for binding to VEGFR-3, and are therefore contemplated as molecules that inhibit VEGF-C-mediated stimulation of VEGFR-3. Because of the ΔC_{156} alteration, such polypeptides (especially covalent or noncovalent dimers of such polypeptides) are not expected to bind VEGFR-2. Thus, certain ΔC_{156} polypeptides and polypeptide dimers are expected to have utility as selective inhibitors of VEGF-C biological activity mediated through VEGFR-3 (i.e., without substantially altering VEGF-C mediated stimulation of VEGFR-2).

In another embodiment of the invention, heterodimers comprising a biologically active VEGF-C polypeptide in association with a ΔC_{156} polypeptide are contemplated. It is contemplated that such heterodimers can be formed *in vitro*, as described below in Example 37, or formed *in vivo* with endogenous VEGF-C following administration of a ΔC_{156} polypeptide. Such heterodimers are contemplated as modulators of VEGF-C mediated effects in cells where the biological effects of VEGF-C are mediated through VEGFR-2/VEGFR-3 heterodimers. VEGF-C ΔC_{156} polypeptides in homodimers or in heterodimers with wt VEGF-C might selectively inhibit the ability of the latter to induce VEGF-like effects, particularly to increase the vascular permeability.

Replacement of the second and/or the fourth of the eight conserved cysteine residues of VEGF abolishes VEGF dimer formation and VEGF biological activity. The analogous effect was investigated for VEGF-C, wherein the cysteines at positions 156 and 165 of SEQ ID NO: 8 correspond to the second and fourth conserved cysteines. No homodimers were obtained when VEGF-CΔNΔCHisC156,165S (i.e., Cys_{156} and Cys_{165} both replaced with serine residues) or in VEGF-CΔNΔCHisC165S were chemically crosslinked. On the other hand, about half of both crosslinked VEGF-CΔNΔCHis and

VEGF-CΔNΔCHisC156S migrated as dimers. This data indicates that VEGF-CΔNΔCHisC156S forms homodimers. Moreover, unlike VEGF-CΔNΔCHis, which forms preferentially non-covalently bound dimers, a fraction of VEGF-CΔNΔCHisC156S was disulfide bonded, as detected by SDS-PAGE in non-reducing conditions. In receptor binding studies (using procedures such as those described above), the C165S and C156,165S forms were both unable to bind VEGFR-3 or VEGFR-2. Collectively, these data suggest that homodimerization is required for VEGFR-3 activation by VEGF-C, and indicate that the inability of ΔNΔC156S to activate VEGFR-2 and to induce VEGF-like effects is not due to an inability of this mutant to form homodimers.

Example 36

Utility for VEGF-C in promoting myelopoiesis

The effects of VEGF-C on hematopoiesis were also analyzed. Specifically, leukocytes populations were analyzed in blood samples taken from the F1 transgenic mice described in Example 29, and from their non-transgenic littermates. Leukocyte population data from these mice and from non-transgenic FVB-NIH control mice (i.e., the strain used to generate the transgenic mice) are set forth in the tables below.

FVB/NIH MICE					
Cell Type	male 5.5 months	male 5.5 months	female 9.5 months	male 9.5 months	mean±σ
Lymphocytes	72.20%	82.17%	84.25%	74.25%	78.22±5.10
Neutrophils	**23.00%**	**15.17%**	**14.25%**	**22.25%**	**18.67±3.98**
Monocytes	0.65%	1.00%	0.25%	0.50%	0.60±0.27
Eosinophils	2.15%	1.70%	1.25%	3.00%	2.03±0.65
Basophils	0.00%	0.00%	0.00%	0.00%	0±0

VEGF-C TRANSGENIC MICE				
Cell Type	male 2 months	male 3.5 months	male 7 months	mean±σ
Lymphocytes	41.3%	41.50%	18.70%	33.83±10.70
Neutrophils	**55.3%**	**53.80%**	**80.17%**	**63.09±12.09**
Monocytes	2.16%	1.30%	0.67%	1.38±0.61
Eosinophils	1.17%	3.50%	.50%	1.72±1.29
Basophils	0.00%	0.00%	0.00%	0±0

VEGF-C NEGATIVE CONTROL MICE (NON-TRANSGENIC LITTERMATES OF VEGF-C TRANSGENIC MICE)					
Cell Type	male 2 months	male 2 months	male 3.5 months	male 7 months	mean±σ
Lymphocytes	89.00%	67.50%	91.00%	71.30%	79.7±10.41
Neutrophils	**7.75%**	**23.00%**	**7.00%**	**23.75%**	**15.38±8.01**
Monocytes	1.50%	0.50%	0.83%	0.75%	0.90±0.37
Eosinophils	1.50%	9.00%	0.67%	4.00%	3.79±3.25
Basophils	0.00%	0.00%	0.50%	0.50%	0.25±0.25

As the foregoing data indicates, the overexpression of VEGF-C in the skin of the transgenic mice correlates with a distinct alteration in leukocyte populations. Notably, the measured populations of neutrophils were markedly increased in the transgenic mice. One explanation for the marked increase in neutrophils is a myelopoietic activity attributable to VEGF-C. A VEGF-C influence on leukocyte trafficking in and out of tissues also may effect observed neutrophil populations. Fluorescence-activated cell sorting analysis, performed on isolated human bone marrow and umbilical cord blood CD34-positive hematopoietic cells, demonstrated that a fraction of these cells are positive for Flt4 (VEGFR-3). Thus, the VEGF-C effect on myelopoiesis may be exerted through this VEGFR-3-positive cell population and its receptors. In any case, the foregoing data indicates a use for VEFG-C polypeptides to increase granulocyte (and, in particular,

neutrophil) counts in human or non-human subjects, i.e., in order to assist the subject fight infectious diseases. The exploitation of the myelopoietic activity of VEGF-C polypeptides is contemplated both *in vitro* (i.e., in cell culture) and *in vivo*, as a sole myelopoietic agent and in combination with other effective agents (e.g., granulocyte colony stimulating factor (G-CSF)).

Additional studies of the myelopoietic effect of VEGF-C, using VEGF-C mutants (e.g., VEGF-C ΔC_{156} polypeptides, VEGF-C ΔNΔCHis, VEGF-C R226,227S) having altered VEGFR-2 binding affinities, will elucidate whether this effect is mediated through VEGFR-2, VEGFR-3, or both receptors, for example. The results of such analysis will be useful in determining which VEGF-C mutants have utility as myelopoietic agents and which have utility as agents for inhibiting myelopoiesis.

Example 37

Generation of Heterodimers consisting of members of the VEGF family of growth factors

Both naturally-occurring and recombinantly-produced heterodimers of polypeptides of the PDGF/VEGF family of growth factors have been shown to exist in nature and possess mitogenic activities. See, e.g., Cao *et al.*, *J. Biol. Chem.*, *271*:3154-62 (1996); and DiSalvo, *et al.*, *J.Biol.Chem.*, *270*:7717-7723 (1995). Heterodimers comprising a VEGF-C polypeptide may be generated essentially as described In Cao *et al.* (1996), using recombinantly produced VEGF-C polypeptides, such as the VEGF-C polypeptides described in the preceding examples. Briefly, a recombinantly produced VEGF-C polypeptide is mixed at an equimolar ratio with another recombinantly produced polypeptide of interest, such as a VEGF, VEGF-B, PlGF, PDGFα, PDGFβ, or *c-fos* induced growth factor polypeptide. (See, e.g., Cao *et al.* (1990); Collins *et al.*, *Nature*, *316*:748-750 (1985) (PDGF-β, GenBank Acc. No. X02811); Claesson-Welsh *et al.*, *Proc. Natl. Acad. Sci. USA*, *86(13)*:4917-4921 (1989) (PDGF-α, GenBank Acc. No. M22734); Claesson-Welsh *et al.*, *Mol. Cell. Biol.* *8*:3476-3486 (1988) (PDGF-β, GenBank Acc. No. M21616); Olofsson *et al.*, *Proc. Natl. Acad. Sci. (USA)*, *93*:2576-2581 (1996) (VEGF-B, GenBank Acc. No. U48801); Maglione *et al.*, *Proc. Natl. Acad. Sci. (USA)*, *88(20)*:9267-9271 (1996) (PlGF, GenBank Acc. No. X54936); Heldin *et al.*, *Growth Factors*, *8*:245-252 (1993); Folkman, *Nature Med.*, *1*:27-31 (1995); Friesel *et al.*, *FASEB J.*, *9*:919-25

(1995); Mustonen *et al., J. Cell. Biol., 129:*895-98 (1995); Orlandini, S., *Proc. Natl. Acad. Sci. USA, 93(21):11675-11680* (1996); and others cited elsewhere herein. The mixed polypeptides are incubated in the presence of guanidine-HCl and DTT. The thiol groups are then protected with S-sulfonation, and the protein is dialyzed overnight, initially against urea/glutathione-SH, glutathione-S-S-glutathione, and subsequently against 20 mM Tris-HCl.

In a preferred embodiment, a variety of differently processed VEGF-C forms and VEGF-C variants and analogs, such as the ones described in the preceding examples, are employed as the VEGF-C polypeptide used to generate such heterodimers. Thereafter, the heterodimers are screened to determine their binding affinity with respect to receptors of the VEGF/PDGF family (especially VEGFR-1, VEGFR-2, and VEGFR-3), and their ability to stimulate the receptors (e.g., assaying for dimer-stimulated receptor phosphorylation in cells expressing the receptor of interest on their surface). The binding assays may be competitive binding assays such as those described herein and in the art. In the initial binding assays, recombinantly produced proteins comprising the extracellular domains of receptors are employable, as described in preceding examples for VEGFR-2 and VEGFR-3. Heterodimers that bind and stimulate receptors are useful as recombinant growth factor polypeptides. Heterodimers that bind but do not stimulate receptors are useful as growth factor antagonists. Heterodimers that display agonistic or antagonistic activities in the screening assays are further screened using, e.g., endothelial cell migration assays, vascular permeability assays, and *in vivo* assays. It will also be apparent from the preceding examples that dimers comprising two VEGF-C polypeptides (i.e., dimers of identical VEGF-C polypeptides as well as dimers of different VEGF-C polypeptides) are advantageously screened for agonistic and antagonistic activities using the same assays.

In one preferred embodiment, VEGF-C ΔC_{156} polypeptide is employed to make the dimers. It is anticipated that agonists and antagonists comprising a VEGF-C ΔC_{156} polypeptide will have increased specificity for stimulating and inhibiting VEGFR-3, without concomitant stimulation or inhibition of VEGFR-2.

In another preferred embodiment, VEGF-C polypeptides wherein the C-terminal proteolytic cleavage site has been altered to reduce or eliminate C-terminal processing (e.g. VEGF-C R226,227S) is employed to make dimers for screening for inhibitory activity.

In yet another preferred embodiment, VEGF-C polypeptides comprising amino-terminal fragments (e.g., the VEGF-C 15 kD form described herein) of VEGF-C are employed to make dimers.

It is further contemplated that inactivation of only one polypeptide chain in a dimer could be enough to generate an inhibitory molecule, which is demonstrated e.g., by the generation of PDGF inhibitory mutant as reported in Vassbotn, *Mol.Cell.Biol.*, *13*:4066-4076 (1993). Therefore, in one embodiment, inhibition is achieved by expression *in vivo* of a polynucleotide (e.g., a cDNA construct) encoding the heterodimerization partner which is unable to bind (or binds inefficiently) to the receptor, or by direct administration of that monomer in a pharmaceutical composition.

Example 38

Formation and Screening of Useful Recombinant VEGF/VEGF-C genes and polypeptides

Amino acid sequence comparison reveals that mature VEGF-C bears structural similarity to VEGF121 [Tischer *et al.*, *J. Biol. Chem.*, *266(18)*:11947-54 (1991)], with certain noteworthy structural differences. For example, mature VEGF-C contains an unpaired cysteine (position 137 of SEQ ID NO: 8) and is able to form non-covalently bonded polypeptide dimers. In one embodiment of the invention, a VEGF analog is created wherein the unpaired cysteine residue from mature VEGF-C is introduced at an analogous position of VEGF (e.g., introduced at Leu_{58} of the human VEGF165 precursor (Fig. 2, Genbank Acc. No. M32977) to generate a $VEGF^{+cys}$ mutant designated VEGF L58C). Such an alteration is introduced into the VEGF165 coding sequence using site-directed mutagenesis procedures known in the art, such as the procedures described above in preceding examples to generate various VEGF-C mutant forms. This $VEGF^{+cys}$ mutant is recombinantly expressed and is screened (alone and as a heterodimer with other VEGF and VEGF-C forms) for VEGFR-2 and/or VEGFR-3 binding, stimulatory, and inhibitory activities, using *in vitro* and *in vivo* activity assays as described elsewhere herein. To form another VEGF analog of the invention, a $VEGF^{+cys}$ mutant is altered to remove a conserved cysteine corresponding to cys_{77} of the VEGF165 precursor. Elimination of this cysteine from the VEGF L58C would result in a VEGF analog resembling VEGF-CΔNΔCHisC156S. This VEGF analog is screened for its

VEGF-inhibitory activities with respect to VEGFR-2 and/or VEGFR-1 and for VEGF-C like stimulatory or inhibitory activities.

Another noteworthy structural difference between VEGF and VEGF-C is the absence in VEGF-C of several basic residues found in VEGF (e.g., residues Arg_{108}, Lys_{110} and His_{112} in the VEGF165 precursor shown in Fig. 2) that have been implicated in VEGF receptor binding. See Keyt *et al., J. Biol. Chem., 271(10)*:5638-46 (1996). In another embodiment of the invention, codons for basic residues (lys, arg, his) are substituted into the VEGF-C coding sequence at one or more analogous positions by site-directed mutagenesis. For example, in a preferred embodiment, Glu_{187}, Thr_{189}, and Pro_{191} in VEGF-C (SEQ ID NO: 8) are replaced with Arg, Lys, and His residues, respectively. The resultant VEGF-C analogs (collectively termed "VEGF-C^{basic}" polypeptides) are recombinantly expressed and screened for VEGFR-1, VEGFR-2, and VEGFR-3 stimulatory and inhibitory activity. The foregoing VEGF and VEGF-C analogs that have VEGF-like activity, VEGF-C-like activity, or that act as inhibitors of VEGF or VEGF-C, are contemplated as additional aspects of the invention. Polynucleotides encoding the analogs also are intended as aspects of the invention.

EXAMPLE 39

EFFECTS OF VEGF-C ON GROWTH AND DIFFERENTIATION OF HUMAN CD34+ PROGENITOR CELLS *IN VITRO*

Human CD34+ progenitor cells (HPC, 10×10^3) were isolated from bone marrow or cord blood mononuclear cells using the MACS CD34 Progenitor cell Isolation Kit (Miltenyi Biotec, Bergish Gladbach, Germany), according to the instructions of the manufacturer and cultured in RPMI 1640 medium supplemented with L-glutamine (2.5 mM), penicillin (125 IE/ml), streptomycin (125 µg/ml) and pooled 10 % umbilical cord blood (CB) plasma at 37 °C in a humidified atmosphere in the presence of 5% CO_2 for seven days, with or without VEGF-C and with or without one of the combinations of growth factors described below. Each experiment was performed in triplicate. After seven days, total cell number was evaluated in each culture.

In a first set of experiments, VEGF-C was added, at concentrations ranging from 10 ng/ml to 1 µg/ml, to the cultures of CB CD34+ HPCs. Cell numbers were evaluated at day 7 of culture. When added as a single factor, 100 ng/ml of VEGF-C was

found support the survival and proliferation of only a few CD34+ HPCs under serum-free conditions. With medium alone, most of the cells died within a culture period of 7 days. However, there were consistently more cells in the cultures provided with the VEGF-C.

A subsequent set of experiments investigated the co-stimulatory effect of VEGF-C in cultures either supplemented with recombinant human stem cell factor (rhSCF, 20 ng/ml PreproTech, Rocky Hill, NY) alone or a combination of granulocyte macrophage colony stimulating factor (rhGM-CSF, 100 ng/ml, Sandoz, Basel, Switzerland) plus SCF. Addition of VEGF-C to SCF-supplemented cultures resulted in a slight co-stimulatory effect on cell growth of CD34+ cells, and this effect was already observable at a VEGF-C concentration of 10 ng/ml. Addition of VEGF-C to GM-CSF- plus SCF-supplemented cultures clearly increased cell yields after 7 days of culture, with an optimum VEGF-C concentration of 100 ng/ml. Additional experiments were conducted to analyze the co-stimulatory effects of 100 ng/ml VEGF-C on total cell yields of serum-free cultures of CB CD34+ HPC cells supplemented with either GM-CSF alone, IL-3 (rhIL-3, 100 U/ml, Behring AG, Marburg, Germany) alone; or a combination of GM-CSF plus IL-3. The results are shown below in the following table:

Total cell number (E x 10^{-3}) after a culture period of 7 days in RPMI + 10% CBPL, + specified growth factors with (+) or without (-) VEGF-C. (Cell number at day 0 = 10)			
Growth Factor(s)	**experiment number**	**- VEGF-C**	**+ VEGF-C**
GM-CSF	1	11	15
	2	10	17
	3	19	25
	mean±SE	**13.3±2.8**	**19.0±3.1***
IL-3	1	113	130
	2	107	113
	3	200	433
	4	45	90
	mean±SE	**116.2±31.9**	**191.5±80.9**
GM-CSF + IL-3	1	150	160
	2	130	140
	3	140	155
	mean±SE	**140.0±5.7**	**151.7±6.0***
GM-CSF + SCF	1	31	37
	2	60	227
	3	47	50
	mean±SE	**46.0±8.3**	**104.7±61.3**

*mean±SE; p=0.02

As depicted in the table, VEGF-C led to a consistent enhancement of cell growth when added as a supplement to each growth factor or combination of growth factors tested.

Effect of VEGF-C on granulomonocytic differentiation of CD34+ progenitors

Using cells from the (7 day) plasma-supplemented cultures described above, immunofluorescence triple stainings were performed to analyze the expression of the early granulomonocytic marker molecules lysozyme (LZ) and myeloperoxidase (MPO) as well as the lipopolysaccharide (LPS) receptor CD14. The table below depicts the percentages and numbers of cells expressing MPO and/or LZ:

Percentages and numbers of cells expressing the markers MPO and LZ after 7 days of culture with (+) or without (-) VEGF-C and specified growth factors						
			percent of cells positive for cell marker		numbers of cells positive for cell marker (E x 10^{-3})	
factor	marker	exp. no.	- VEGF-C	+ VEGF-C	- VEGF-C	+ VEGF-C
GM-CSF	MPO	1	57	69	6	11
		2	45	53	5	9
		3	18	24	10	13
		mean±SE	**40.0±11.0**	**49.0±13***	**7.0±1.5**	**11.0±1.5***
	LZ	1	54	70	6	11
		2	16	16	2	3
		3	15	23	9	13
		mean±SE	**28.0±12.8**	**36.0±16.7**	**5.7±2.0**	**9.0±3.0**
IL-3	MPO	1	20	28	23	36
		2	37	42	39	48
		3	5	9	10	35
		mean±SE	**21.0±9.0**	**26.0±9.0**	**24.0±8.3**	**39.7±4.2**
	LZ	1	15	22	17	29
		2	3	3	3	3
		3	3	5	6	22
		mean±SE	**7.0±4.0**	**10.3±5.8**	**8.7±4.0**	**18.0±7.0**
GM-CSF + IL-3	MPO	1	29	37	46	56
		2	38	40	49	56
		3	6	10	3	6
		mean±SE	**24.0±9.0**	**39.3±16.6**	**32.7±14.8**	**39.3±16.6**
	LZ	1	18	20	29	30
		2	2	3	3	3
		3	1	2	1	2
		mean±SE	**7.0±5.5**	**8.3±5.8**	**11.0±9.0**	**12.0±9.0**
GM-CSF + SCF	MPO	1	50	51	15	19
		2	16	21	10	48
		mean±SE	**33.0±17.0**	**36.0±15.0**	**12.5±2.5**	**33.5±14.5**
	LZ	1	15	15	5	6
		2	9	20	5	45
		mean±SE	**12.0±3.0**	**18.0±2.0**	**5.0±0.0**	**25.5±19.5**

Among the granulomonocytic markers tested, VEGF-C led to an increase in the proportion of LZ+ cells under all culture conditions. In comparison, LZ+CD14+ cells, which represent differentiated monocytic cells only very slightly increased upon addition of VEGF-C (data not shown). Co-stimulation of the cells with VEGF-C increased the expression of MPO, an early granulocytic marker molecule, only modestly, except in combination with both GM-CSF and IL-3, where the increase in the proportion of MPO+ cells was more pronounced.

VEGF-C exerts co-stimulatory effects in combination with M-CSF

In another series of experiments, CD34+ cells were cultured in medium supplemented with 50 ng/ml M-CSF, with or without 100 ng/ml VEGF-C, for seven days. Culture of CD34+ cells in the presence of M-CSF leads to the generation of CD14+ monocytes within 7 days. After seven days, the cultures were analyzed to determine the percentages of CD14+ cells and mean fluorescence intensity. The results are summarized in the table below:

Percentages of $CD14^+$ cells and mean fluorescence intensity (MFI) of cells cultured with M-CSF in the absence or in the presence of VEGF-C

	M-CSF alone		M-CSF + VEGF-C	
exp no	**% CD14+**	**MFI**	**% CD14+**	**MFI**
1	37	20	47	40
2	42	44	54	74
3	32	6	36	7
mean±SE	**36.8±2.9**	**23.3±11.1**	**45.7±5.2**	**40.3±19.3**

As shown in the table, addition of VEGF-C to these cultures increased both the proportion of CD14+ cells (37% CD14+ cells vs. 46%) and the fluorescence intensity of CD14 expression (MFI 23.3 vs. 40.3). However, cell numbers did not increase upon addition of VEGF-C to M-CSF supplemented cultures. Thus, VEGF-C had a small effect on the differentiation of monocytic cells, but not on their growth.

In the foregoing experiments the presence of VEGF-C was associated with enhanced numbers of cells in cultures of cord blood CD34+ cells. Under all conditions tested (GM-CSF, IL-3, GM-CSF + IL-3; GM-CSF + SCF), co-culture with VEGF-C led

to an enhancement of proportions of myeloid cells. These results indicate an application for VEGF-C in the stimulation and/or differentiation of CD34+ progenitor cells *in vitro* or *in vivo*. Furthermore, the use of VEGF-C alone also slightly increased the number of surviving cells. The results thus indicate uses for compositions comprising VEGF-C prepared in admixture with the aforementioned or other growth factors, such as VEGF-C, and unit dose formulations comprising VEGF-C packaged together with the aforementioned or other growth factors. Such compositions, unit dose formulations, and methods of their use are intended as further aspects of the present invention.

Deposit of Biological Materials: Plasmid FLT4-L has been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville MD 20952 (USA), pursuant to the provisions of the Budapest Treaty, and has been assigned a deposit date of 24 July 1995 and ATCC accession number 97231.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those in the art. Accordingly, only such limitations as appear in the appended claims should be placed on the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Ludwig Institute for Cancer Research
Helsinki University Licensing
Alitalo, Kari(U.S. only)
Joukov, Vladimir(U.S. only)

(ii) TITLE OF INVENTION: Vascular Endothelial Growth Factor C (VEGF-C) Protein and Gene, Mutants Thereof, and Uses Thereof

(iii) NUMBER OF SEQUENCES: 59

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606-6402

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:08/795,430
(B) FILING DATE: 05-FEB-1997

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/FI96/00427
(B) FILING DATE: 01-AUG-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/671,573
(B) FILING DATE: 28-JUN-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/601,132
(B) FILING DATE: 14-FEB-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/585,895
(B) FILING DATE: 12-JAN-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/510,133
(B) FILING DATE: 01-AUG-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/340,011
(B) FILING DATE: 14-NOV-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Gass, David A.
(B) REGISTRATION NUMBER: 38,153
(C) REFERENCE/DOCKET NUMBER: 28967/34140

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312/474-6300
(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4416 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCACGCGCAG CGGCCGGAGA TGCAGCGGGG CGCCGCGCTG TGCCTGCGAC TGTGGCTCTG 60

CCTGGGACTC CTGGACGGCC TGGTGAGTGG CTACTCCATG ACCCCCCCGA CCTTGAACAT 120

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GCACCCCCTC GAGTGGGCTT GGCCAGGAGC TCAGGAGGCG CCAGCCACCG GAGACAAGGA 240

CAGCGAGGAC ACGGGGGTGG TGCGAGACTG CGAGGGCACA GACGCCAGGC CCTACTGCAA 300

GGTGTTGCTG CTGCACGAGG TACATGCCAA CGACACAGGC AGCTACGTCT GCTACTACAA 360

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CTTTGAGCAG CCATTCATCA ACAAGCCTGA CACGCTCTTG GTCAACAGGA AGGACGCCAT 480

GTGGGTGCCC TGTCTGGTGT CCATCCCCGG CCTCAATGTC ACGCTGCGCT CGCAAAGCTC 540

GGTGCTGTGG CCAGACGGGC AGGAGGTGGT GTGGGATGAC CGGCGGGGCA TGCTCGTGTC 600

CACGCCACTG CTGCACGATG CCCTGTACCT GCAGTGCGAG ACCACCTGGG GAGACCAGGA 660

CTTCCTTTCC AACCCCTTCC TGGTGCACAT CACAGGCAAC GAGCTCTATG ACATCCAGCT 720

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CGTCGAGTGG CTCAAAGGAC CCATCCTGGA GGCCACGGCA GGAGACGAGC TGGTGAAGCT 1080

GCCCGTGAAG CTGGCAGCGT ACCCCCCGCC CGAGTTCCAG TGGTACAAGG ATGGAAAGGC 1140

ACTGTCCGGG CGCCACAGTC CACATGCCCT GGTGCTCAAG GAGGTGACAG AGGCCAGCAC 1200

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CCTGTCCACG CTGCACGATG CGCACGGGAA CCCGCTTCTG CTCGACTGCA AGAACGTGCA 1860

TCTGTTCGCC ACCCCTCTGG CCGCCAGCCT GGAGGAGGTG GCACCTGGGG CGCGCCACGC 1920

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 216 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAAGAAAGCG GCTTCAGCTG TAAAGGACCT GGCCAGAATG TGGCTGTGAC CAGGGCACAC 60

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TACAACAGCG AGTATGGGGA GCTGTCGGAG CCAAGCGAGG AGGACCACTG CTCCCCGTCT 180

GCCCGCGTGA CTTTCTTCAC AGACAACAGC TACTAA 216

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4273 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG 2880

CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG 2940

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CCAATTTGTT AAAGACAGGA TATCAGTGGT CCAGGCTCTA GTTTTGACTC AACAATATCA 3720

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CTGAGTCGCC CGG 4273

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
1 5 10 15

Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
20 25 30

His Arg Gln Glu Ser Gly Phe Arg
35 40

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
1 5 10 15

Leu Lys

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCACTATAGG GAGACCCAAG CTTGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT 60

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TGTCAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG 180

ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC 219

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1997 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCCGCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCCTCGCC 60

CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCCGGTTT CCTGTGAGGC 120

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GGAACGCGGA GCCCCGGACC CGCTCCCGCC GCCTCCGGCT CGCCCAGGGG GGGTCGCCGG 240

GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCGCGCCC 300

CCACCCCTGC CCCCGCCAGC GGACCGGTCC CCCACCCCCG GTCCTTCCAC C ATG CAC 357
Met His
1

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Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala
 35                  40                  45                  50

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Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val
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Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn
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Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr
     100                 105                 110

AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA        741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln
115                 120                 125                 130

TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC        789
Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val
                135                 140                 145

GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT        837
Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys
            150                 155                 160

GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG        885
Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr
        165                 170                 175

AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA        933
Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln
    180                 185                 190

GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA        981
Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg
195                 200                 205                 210

TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA       1029
Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg
                215                 220                 225
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```
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC      1077
Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr
            230                 235                 240

TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT      1125
Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala
        245                 250                 255

CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT      1173
Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp
    260                 265                 270

GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC      1221
Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr
275                 280                 285                 290

TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC      1269
Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro
                295                 300                 305

CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA      1317
His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys
            310                 315                 320

CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA      1365
Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr
        325                 330                 335

TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT      1413
Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn
    340                 345                 350

CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG      1461
Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu
355                 360                 365                 370

TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG      1509
Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg
                375                 380                 385

CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT      1557
Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser
            390                 395                 400

GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG      1605
Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met
        405                 410                 415

AGC TAAGATTGTA CTGTTTTCCA GTTCATCGAT TTTCTATTAT GGAAAACTGT           1658
Ser

GTTGCCACAG TAGAACTGTC TGTGAACAGA GAGACCCTTG TGGGTCCATG CTAACAAAGA   1718

CAAAAGTCTG TCTTTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCATC   1778

TGCAAAAGGC CTCTTGTAAA GACTGGTTTT CTGCCAATGA CCAAACAGCC AAGATTTTCC   1838
```

TCTTGTGATT TCTTTAAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT 1898

GCATTCATTT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTTATATC 1958

ATGCAAAATA TGTTTAAAAT AAAATGAAAA TTGTATTAT 1997

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 419 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
1 5 10 15

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe
20 25 30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
35 40 45

Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
50 55 60

Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
65 70 75 80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
85 90 95

Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
100 105 110

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
115 120 125

Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
130 135 140

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
145 150 155 160

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
165 170 175

Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
180 185 190

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
195 200 205

```
Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
    210                 215                 220

Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
225                 230                 235                 240

Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
                245                 250                 255

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
            260                 265                 270

Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
        275                 280                 285

Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
    290                 295                 300

Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
305                 310                 315                 320

Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
                325                 330                 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
            340                 345                 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
        355                 360                 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
    370                 375                 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385                 390                 395                 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
                405                 410                 415

Gln Met Ser
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu
1 5 10 15

Lys

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1836 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 168..1412

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCGGCCGCGT CGACGCAAAA GTTGCGAGCC GCCGAGTCCC GGGAGACGCT CGCCCAGGGG 60

GGTCCCCGGG AGGAAACCAC GGGACAGGGA CCAGGAGAGG ACCTCAGCCT CACGCCCCAG 120

CCTGCGCCAG CCAACGGACC GGCCTCCCTG CTCCCGGTCC ATCCACC ATG CAC TTG 176
Met His Leu
1

CTG TGC TTC TTG TCT CTG GCG TGT TCC CTG CTC GCC GCT GCG CTG ATC 224
Leu Cys Phe Leu Ser Leu Ala Cys Ser Leu Leu Ala Ala Ala Leu Ile
5 10 15

CCC AGT CCG CGC GAG GCG CCC GCC ACC GTC GCC GCC TTC GAG TCG GGA 272
Pro Ser Pro Arg Glu Ala Pro Ala Thr Val Ala Ala Phe Glu Ser Gly
20 25 30 35

CTG GGC TTC TCG GAA GCG GAG CCC GAC GGG GGC GAG GTC AAG GCT TTT 320
Leu Gly Phe Ser Glu Ala Glu Pro Asp Gly Gly Glu Val Lys Ala Phe
40 45 50

GAA GGC AAA GAC CTG GAG GAG CAG TTG CGG TCT GTG TCC AGC GTA GAT 368
Glu Gly Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp
55 60 65

GAG CTG ATG TCT GTC CTG TAC CCA GAC TAC TGG AAA ATG TAC AAG TGC 416
Glu Leu Met Ser Val Leu Tyr Pro Asp Tyr Trp Lys Met Tyr Lys Cys
70 75 80

CAG CTG CGG AAA GGC GGC TGG CAG CAG CCC ACC CTC AAT ACC AGG ACA 464
Gln Leu Arg Lys Gly Gly Trp Gln Gln Pro Thr Leu Asn Thr Arg Thr
85 90 95

```
GGG GAC AGT GTA AAA TTT GCT GCT GCA CAT TAT AAC ACA GAG ATC CTG        512
Gly Asp Ser Val Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu
100                 105                 110                 115

AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGT GAG        560
Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu
                120                 125                 130

GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GCA GCC ACA AAC ACC TTC        608
Val Cys Ile Asp Val Gly Lys Glu Phe Gly Ala Ala Thr Asn Thr Phe
            135                 140                 145

TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAC        656
Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn
        150                 155                 160

AGC GAG GGG CTG CAG TGC ATG AAC ACC AGC ACA GGT TAC CTC AGC AAG        704
Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Gly Tyr Leu Ser Lys
    165                 170                 175

ACG TTG TTT GAA ATT ACA GTG CCT CTC TCA CAA GGC CCC AAA CCA GTC        752
Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val
180                 185                 190                 195

ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGG TGC ATG TCT AAA CTG        800
Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu
                200                 205                 210

GAT GTT TAC AGA CAA GTT CAT TCA ATT ATT AGA CGT TCT CTG CCA GCA        848
Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala
            215                 220                 225

ACA TTA CCA CAG TGT CAG GCA GCT AAC AAG ACA TGT CCA ACA AAC TAT        896
Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr
        230                 235                 240

GTG TGG AAT AAC TAC ATG TGC CGA TGC CTG GCT CAG CAG GAT TTT ATC        944
Val Trp Asn Asn Tyr Met Cys Arg Cys Leu Ala Gln Gln Asp Phe Ile
    245                 250                 255

TTT TAT TCA AAT GTT GAA GAT GAC TCA ACC AAT GGA TTC CAT GAT GTC        992
Phe Tyr Ser Asn Val Glu Asp Asp Ser Thr Asn Gly Phe His Asp Val
260                 265                 270                 275

TGT GGA CCC AAC AAG GAG CTG GAT GAA GAC ACC TGT CAG TGT GTC TGC       1040
Cys Gly Pro Asn Lys Glu Leu Asp Glu Asp Thr Cys Gln Cys Val Cys
                280                 285                 290

AAG GGG GGG CTT CGG CCA TCT AGT TGT GGA CCC CAC AAA GAA CTA GAT       1088
Lys Gly Gly Leu Arg Pro Ser Ser Cys Gly Pro His Lys Glu Leu Asp
            295                 300                 305

AGA GAC TCA TGT CAG TGT GTC TGT AAA AAC AAA CTT TTC CCT AAT TCA       1136
Arg Asp Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Asn Ser
        310                 315                 320
```

```
TGT GGA GCC AAC AGG GAA TTT GAT GAG AAT ACA TGT CAG TGT GTA TGT      1184
Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys
    325                 330                 335

AAA AGA ACG TGT CCA AGA AAT CAG CCC CTG AAT CCT GGG AAA TGT GCC      1232
Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala
340                 345                 350                 355

TGT GAA TGT ACA GAA AAC ACA CAG AAG TGC TTC CTT AAA GGG AAG AAG      1280
Cys Glu Cys Thr Glu Asn Thr Gln Lys Cys Phe Leu Lys Gly Lys Lys
                360                 365                 370

TTC CAC CAT CAA ACA TGC AGT TGT TAC AGA AGA CCG TGT GCG AAT CGA      1328
Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Ala Asn Arg
            375                 380                 385

CTG AAG CAT TGT GAT CCA GGA CTG TCC TTT AGT GAA GAA GTA TGC CGC      1376
Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu Val Cys Arg
        390                 395                 400

TGT GTC CCA TCG TAT TGG AAA AGG CCA CAT CTG AAC TAAGATCATA          1422
Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn
    405                 410                 415

CCAGTTTTCA GTCAGTCACA GTCATTTACT CTCTTGAAGA CTGTTGGAAC AGCACTTAGC   1482

ACTGTCTATG CACAGAAAGA CTCTGTGGGA CCACATGGTA ACAGAGGCCC AAGTCTGTGT   1542

TTATTGAACC ATGTGGATTA CTGCGGGAGA GGACTGGCAC TCATGTGCAA AAAAAACCTC   1602

TTCAAAGACT GGTTTTCTGC CAGGGACCAG ACAGCTGAGG TTTTTCTCTT GTGATTTAAA   1662

AAAAGAATGA CTATATAATT TATTTCCACT AAAAATATTG TTCCTGCATT CATTTTTATA   1722

GCAATAACAA TTGGTAAAGC TCACTGTGAT CAGTATTTTT ATAACATGCA AAACTATGTT   1782

TAAAATAAAA TGAAAATTGT ATTATAAAAA AAAAAAAAAA AAAAAAAAAA GCTT         1836
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 415 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Met His Leu Leu Cys Phe Leu Ser Leu Ala Cys Ser Leu Leu Ala Ala
 1               5                  10                  15

Ala Leu Ile Pro Ser Pro Arg Glu Ala Pro Ala Thr Val Ala Ala Phe
            20                  25                  30
```

```
Glu Ser Gly Leu Gly Phe Ser Glu Ala Glu Pro Asp Gly Gly Glu Val
          35                  40                  45

Lys Ala Phe Glu Gly Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
      50                  55                  60

Ser Val Asp Glu Leu Met Ser Val Leu Tyr Pro Asp Tyr Trp Lys Met
 65                  70                  75                  80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln Gln Pro Thr Leu Asn
                 85                  90                  95

Thr Arg Thr Gly Asp Ser Val Lys Phe Ala Ala Ala His Tyr Asn Thr
            100                 105                 110

Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met
        115                 120                 125

Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Ala Ala Thr
    130                 135                 140

Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly
145                 150                 155                 160

Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Gly Tyr
                165                 170                 175

Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro
            180                 185                 190

Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met
        195                 200                 205

Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser
    210                 215                 220

Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro
225                 230                 235                 240

Thr Asn Tyr Val Trp Asn Asn Tyr Met Cys Arg Cys Leu Ala Gln Gln
                245                 250                 255

Asp Phe Ile Phe Tyr Ser Asn Val Glu Asp Asp Ser Thr Asn Gly Phe
            260                 265                 270

His Asp Val Cys Gly Pro Asn Lys Glu Leu Asp Glu Asp Thr Cys Gln
        275                 280                 285

Cys Val Cys Lys Gly Gly Leu Arg Pro Ser Ser Cys Gly Pro His Lys
    290                 295                 300

Glu Leu Asp Arg Asp Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe
305                 310                 315                 320

Pro Asn Ser Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln
                325                 330                 335
```

```
Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly
            340                 345                 350

Lys Cys Ala Cys Glu Cys Thr Glu Asn Thr Gln Lys Cys Phe Leu Lys
        355                 360                 365

Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys
    370                 375                 380

Ala Asn Arg Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu
385                 390                 395                 400

Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn
                405                 410                 415
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1741 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 453..1706

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
GCCCCCGCCG AGCGCTCCGC GCGCAGCCGC CGGGCCGGGC CGGCCCGCGG AGGGCGCGCT      60

GCGAGCGGCC ACTGGGTCCT GCTTCCCTCC TTCCTCTCCC TCCTCCTCCT CCTCCTTCTC     120

TCTGCGCTTT CCACCGCTCC CGAGCGAGCG CACGCTCGGA TGTCCGGTTT CCTGGTGGGT     180

TTTTTACCTG GCAAAGTCCG GATAACTTCG GTGAGAATTT GCAAAGAGGC TGGGAGCTCC     240

CCTGCAGGCG TCTGGGAGCT GCTGCCGCCG TCGCATCTTC TCCATCCCGC GGATTTTACT     300

GCCTTGGATA TTGCGAGGGG AGGGAGGGGG GTGAGGACAG CAAAAAGAAA GGGGTGGGGG     360

GGGGGAGAGA AAAGGAAAAG AAGGAGCCTC GGAATTGTGC CCGCATTCCT GCGCTGCCCC     420

GCGGCCCCCC TCCGCTCTGC CATCTCCGCA CA ATG CAC TTG CTG GAG ATG CTC        473
                                   Met His Leu Leu Glu Met Leu
                                   1               5

TCC CTG GGC TGC TGC CTC GCT GCT GGC GCC GTG CTC CTG GGA CCC CGG        521
Ser Leu Gly Cys Cys Leu Ala Ala Gly Ala Val Leu Leu Gly Pro Arg
        10                  15                  20
```

```
CAG CCG CCC GTC GCC GCC GCC TAC GAG TCC GGG CAC GGC TAC TAC GAG          569
Gln Pro Pro Val Ala Ala Ala Tyr Glu Ser Gly His Gly Tyr Tyr Glu
    25                  30                  35

GAG GAG CCC GGT GCC GGG GAA CCC AAG GCT CAT GCA AGC AAA GAC CTG          617
Glu Glu Pro Gly Ala Gly Glu Pro Lys Ala His Ala Ser Lys Asp Leu
40                  45                  50                  55

GAA GAG CAG TTG CGA TCT GTG TCC AGT GTG GAT GAA CTC ATG ACA GTA          665
Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val
                60                  65                  70

CTT TAC CCA GAA TAC TGG AAA ATG TTC AAA TGT CAG TTG AGG AAA GGA          713
Leu Tyr Pro Glu Tyr Trp Lys Met Phe Lys Cys Gln Leu Arg Lys Gly
            75                  80                  85

GGT TGG CAA CAC AAC AGG GAA CAC TCC AGC TCT GAT ACA AGA TCA GAT          761
Gly Trp Gln His Asn Arg Glu His Ser Ser Ser Asp Thr Arg Ser Asp
        90                  95                  100

GAT TCA TTG AAA TTT GCC GCA GCA CAT TAT AAT GCA GAG ATC CTG AAA          809
Asp Ser Leu Lys Phe Ala Ala Ala His Tyr Asn Ala Glu Ile Leu Lys
    105                 110                 115

AGT ATT GAT ACT GAA TGG AGA AAA ACC CAG GGC ATG CCA CGT GAA GTG          857
Ser Ile Asp Thr Glu Trp Arg Lys Thr Gln Gly Met Pro Arg Glu Val
120                 125                 130                 135

TGT GTG GAT TTG GGG AAA GAG TTT GGA GCA ACT ACA AAC ACC TTC TTT          905
Cys Val Asp Leu Gly Lys Glu Phe Gly Ala Thr Thr Asn Thr Phe Phe
                140                 145                 150

AAA CCC CCG TGT GTA TCC ATC TAC AGA TGT GGA GGT TGC TGC AAT AGT          953
Lys Pro Pro Cys Val Ser Ile Tyr Arg Cys Gly Gly Cys Cys Asn Ser
            155                 160                 165

GAA GGA CTC CAG TGT ATG AAT ATC AGC ACA AAT TAC ATC AGC AAG ACA         1001
Glu Gly Leu Gln Cys Met Asn Ile Ser Thr Asn Tyr Ile Ser Lys Thr
        170                 175                 180

TTG TTT GAG ATT ACA GTG CCT CTC TCT CAT GGC CCC AAA CCT GTA ACA         1049
Leu Phe Glu Ile Thr Val Pro Leu Ser His Gly Pro Lys Pro Val Thr
    185                 190                 195

GTC AGT TTT GCC AAT CAC ACG TCC TGC CGA TGC ATG TCT AAG TTG GAT         1097
Val Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp
200                 205                 210                 215

GTT TAC AGA CAA GTT CAT TCT ATC ATA AGA CGT TCC TTG CCA GCA ACA         1145
Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr
                220                 225                 230

CAA ACT CAG TGT CAT GTG GCA AAC AAG ACC TGT CCA AAA AAT CAT GTC         1193
Gln Thr Gln Cys His Val Ala Asn Lys Thr Cys Pro Lys Asn His Val
            235                 240                 245
```

```
TGG AAT AAT CAG ATT TGC AGA TGC TTA GCA CAG CAC GAT TTT GGT TTC      1241
Trp Asn Asn Gln Ile Cys Arg Cys Leu Ala Gln His Asp Phe Gly Phe
        250                 255                 260

TCT TCT CAC CTT GGA GAT TCT GAC ACA TCT GAA GGA TTC CAT ATT TGT      1289
Ser Ser His Leu Gly Asp Ser Asp Thr Ser Glu Gly Phe His Ile Cys
    265                 270                 275

GGG CCC AAC AAA GAG CTG GAT GAA GAA ACC TGT CAA TGC GTC TGC AAA      1337
Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Lys
280                 285                 290                 295

GGA GGT GTG CGG CCC ATA AGC TGT GGC CCT CAC AAA GAA CTA GAC AGG      1385
Gly Gly Val Arg Pro Ile Ser Cys Gly Pro His Lys Glu Leu Asp Arg
                300                 305                 310

GCA TCA TGT CAG TGC ATG TGC AAA AAC AAA CTG CTC CCC AGT TCC TGT      1433
Ala Ser Cys Gln Cys Met Cys Lys Asn Lys Leu Leu Pro Ser Ser Cys
            315                 320                 325

GGG CCT AAC AAA GAA TTT GAT GAA GAA AAG TGC CAG TGT GTA TGT AAA      1481
Gly Pro Asn Lys Glu Phe Asp Glu Glu Lys Cys Gln Cys Val Cys Lys
        330                 335                 340

AAG ACC TGT CCC AAA CAT CAT CCA CTA AAT CCT GCA AAA TGC ATC TGC      1529
Lys Thr Cys Pro Lys His His Pro Leu Asn Pro Ala Lys Cys Ile Cys
    345                 350                 355

GAA TGT ACA GAA TCT CCC AAT AAA TGT TTC TTA AAA GGA AAA AGA TTT      1577
Glu Cys Thr Glu Ser Pro Asn Lys Cys Phe Leu Lys Gly Lys Arg Phe
360                 365                 370                 375

CAT CAC CAG ACA TGC AGT TGT TAC AGA CCA CCA TGT ACA GTC CGA ACG      1625
His His Gln Thr Cys Ser Cys Tyr Arg Pro Pro Cys Thr Val Arg Thr
                380                 385                 390

AAA CGC TGT GAT GCT GGA TTT CTG TTA GCT GAA GAA GTG TGC CGC TGT      1673
Lys Arg Cys Asp Ala Gly Phe Leu Leu Ala Glu Glu Val Cys Arg Cys
            395                 400                 405

GTA CGC ACA TCT TGG AAA AGA CCA CTT ATG AAT TAAGCGAAGA AAGCACTACT    1726
Val Arg Thr Ser Trp Lys Arg Pro Leu Met Asn
        410                 415

CGCTATATAG TGTCG                                                    1741
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 418 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met His Leu Leu Glu Met Leu Ser Leu Gly Cys Cys Leu Ala Ala Gly
1 5 10 15

Ala Val Leu Leu Gly Pro Arg Gln Pro Pro Val Ala Ala Ala Tyr Glu
20 25 30

Ser Gly His Gly Tyr Tyr Glu Glu Glu Pro Gly Ala Gly Glu Pro Lys
35 40 45

Ala His Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser
50 55 60

Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Phe
65 70 75 80

Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu His Ser
85 90 95

Ser Ser Asp Thr Arg Ser Asp Asp Ser Leu Lys Phe Ala Ala Ala His
100 105 110

Tyr Asn Ala Glu Ile Leu Lys Ser Ile Asp Thr Glu Trp Arg Lys Thr
115 120 125

Gln Gly Met Pro Arg Glu Val Cys Val Asp Leu Gly Lys Glu Phe Gly
130 135 140

Ala Thr Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Ile Tyr Arg
145 150 155 160

Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Ile Ser
165 170 175

Thr Asn Tyr Ile Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser
180 185 190

His Gly Pro Lys Pro Val Thr Val Ser Phe Ala Asn His Thr Ser Cys
195 200 205

Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile
210 215 220

Arg Arg Ser Leu Pro Ala Thr Gln Thr Gln Cys His Val Ala Asn Lys
225 230 235 240

Thr Cys Pro Lys Asn His Val Trp Asn Asn Gln Ile Cys Arg Cys Leu
245 250 255

Ala Gln His Asp Phe Gly Phe Ser Ser His Leu Gly Asp Ser Asp Thr
260 265 270

Ser Glu Gly Phe His Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu
275 280 285

```
Thr Cys Gln Cys Val Cys Lys Gly Gly Val Arg Pro Ile Ser Cys Gly
    290                 295                 300

Pro His Lys Glu Leu Asp Arg Ala Ser Cys Gln Cys Met Cys Lys Asn
305                 310                 315                 320

Lys Leu Leu Pro Ser Ser Cys Gly Pro Asn Lys Glu Phe Asp Glu Glu
                325                 330                 335

Lys Cys Gln Cys Val Cys Lys Lys Thr Cys Pro Lys His His Pro Leu
            340                 345                 350

Asn Pro Ala Lys Cys Ile Cys Glu Cys Thr Glu Ser Pro Asn Lys Cys
        355                 360                 365

Phe Leu Lys Gly Lys Arg Phe His His Gln Thr Cys Ser Cys Tyr Arg
    370                 375                 380

Pro Pro Cys Thr Val Arg Thr Lys Arg Cys Asp Ala Gly Phe Leu Leu
385                 390                 395                 400

Ala Glu Glu Val Cys Arg Cys Val Arg Thr Ser Trp Lys Arg Pro Leu
                405                 410                 415

Met Asn
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Ala Val Val Met Thr Gln Thr Pro Ala Ser
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCTCTTCTGT GCTTGAGTTG AG 22

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCTCTTCTGT CCCTGAGTTG AG 22

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGTGCTGCAG CAAATTTTAT AGTCTCTTCT GTGGCGGCGG CGGCGGCGGG CGCCTCGCGA 60

GGACC 65

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTGGCAGGGA ACTGCTAATA ATGGAATGAA 30

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGCTCCGCG TCCGAGAGGT CGAGTCCGGA CTCGTGATGG TGATGGTGAT GGGCGGCGGC 60

GGCGGCGGGC GCCTCGCGAG GACC 84

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTATTATAAT GTCCTCCACC AAATTTTATA G 31

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTTCGCTGCC TGACACTGTG GTAGTGTTGC TGGCGGCCGC TAGTGATGGT GATGGTGATG 60

AATAATGGAA TGAACTTGTC TGTAAACATC CAG 93

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CATGTACGAA CCGCCAGG 18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AATGACCAGA GAGAGGCGAG 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCCACGGTAG GTCTGCGT 18

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTTCTTTGAC AGGCTTAT 18

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATCTTGAAAA GTAAGTATGG G 21

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGACTTGAC AGGTATTGAT 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGCAAGACGG TGGGTATTGT 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTTCTTTG TAGTTATTTG AA 22

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCACAGTGAG TATGAATTAA 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TTCTTCCAAA GGTGTCAG 18

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGAGATGGTA GCAGAATG 18

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CTATTTGTCT AGACTCAACA GAT 23

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CAAACATGCA GGTAAGAGAT CC 22

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGTTCTCCTA GCTGTTACAG A 21

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGCGAGGTCA AGGTAGGTGC AAGG 24

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ATTGTCTTTG ACAGGCTTTT TGAAGG 26

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GAGATCCTGA AAAGTAAGTA G 21

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

TGTGACTCGA CAGGTATTGA TAAT 24

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CTCAGCAAGA CGGTAGGTAT 20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TTGTCCCTTG TAGTTGTTTG AAATT 25

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ACATTACCAC AGTGAGTATG 20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GTCTCCCCAA AAGGTGTCAG GCAGCT 26

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AATGTTGAAG ATGGTAAGTA AAA 23

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

TCTAGACTCA ACCAAT 16

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CAAACATGCA GGTAAGGAGT GT 22

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TTTTCCCCTA GTTGTTACAG AAGA 24

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2991 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GTTTTAAGTA GAGACGGGGT TTCACCAACG GTTGAAAATA TTTATCATGG TCTCCCTAAG 60

ATGGACGGTG TTAGCTAGGA TGGTCTCGAT CTCCTGACCT CATGATCCAC CCGCCTCGGC 120

CTCCCAAAGT GCTGGGATTA CAGGCGTGAG CCACCGTGTC CGACCAACCT TAAGACAAAC 180

AACTACTGCA TGATTGTTTT TGGAGACCTT TTTTTTATTC AAATAAATTT TTGCCAGCAT 240

TTTCTGACTC AAAGTATAGC AGCAGGAAGA TAACACTTTT GTGAGAAAAA AGTTTGAATA 300

CAGCTTACTG CTGTATTTAA ATGAAACAGT AGTTAATATG ATATTAATAT ATTTTGGATA 360

TATTTTGAGT TTGTTGATTT TCCAGTCTTC ACCCGCTGCT AGGCCTGTGG GTGTTGGAAA 420

TGCCTGTGTT TCTCAATTTT GTTTGCCTAT TAGAATCCTG ATGTCCAAGC CTTACTCCAG 480

TTAGACCAGT TAAGCCAGAA AGGCAGAAGG TGTACTCAAG CATCTGTTTT TTCAAAATCT 540

CCTTTTGTGA TGCCAAGTGC AATCAAAGTT TAGAATCATT GTAATAGCAA ATGGTTGAAT 600

GGAAACTCCA CCTTCTATTC AAATCCTACC CCAGTCTGCC CTTAGCTGTT CTCTTTTCAC 660

AGATCTATCA ATGTCTGAAG ATAACTATGG CAGGCTGATC AAATATGCAT AGAGCAGGAA 720

GACAGCAAGA GAGTGATACA CTGACCATGT TCCAAATCAC AAAACATCTC AACAGGCTAG 780

ATCATGGACC GAGTCTGATG GGATGGAATT TCATAAAGAT ACATAAAAAA GCATCTTGGA 840

TACAGTAAAC TTAACTCCAC AAATACAGGG GAATTTAGAC GTGACTAAGT AGCAGTACAT 900

ATGAAAAATT ATTGAGGAAT TTTGTTGACT TTAAGGGTAG TGTGAGTCAA CACTGTGATT 960

TGGCTGCCAG AAAATAAACT CAATCCAAGG CTGTATCAAC AAAGGCATAC TGTCCATTCT 1020

GCATGCTCAT TACAGCACTA AGTACCGAGC CATGTTCTCA ACCGCATACT TCATGAACAT 1080

GGAAAGCTAA CAGTATGGTT AAGGGGGGAA ACTGGAACTG TCATCTTGGG GAATAAAAGG 1140

GATATTTAGC CAGGAGTAAA GTTAGCTTAG GGAGACCATG ATAAATATTT TCAAAATATT 1200

TGAAGGACTC AGTTGTGGAA GTGAGATTAG ATTTATTGTG TAAAACTCCA GGAGTCAAAA 1260

GCAATAGAGA GATAGAAGGA AATGCTTTTC AGCAGTGTTG CTCATCAATA AAGGGAGTGA 1320

ACAGCCACAC AGAATGGAAG GTTCCCTGTC CTTTGAGATA TTTAAGCCTT CAAGTAAATT 1380

ATGGGTGAGG AGTTTCAAAT CTAGAGTTGA ACCAGATAAG AAAGTCTCTT CTTCCGGTAA 1440

GATATTATGG ACCTATAACA TCTGTGTACT TAAAAGTAGA TTGGGAGTGA AAGGCAGACT 1500

TTTGATGTTC TGTACACTGT TGAAACCCCT TAGCGTGGTC CTCTGTAACC TGCTCACCCT 1560

GCCCCAAGGA GGCAGCTAGC CAATGCCACC AGCCCAACGG AAACCCCAGT GCTTTTCCAA 1620

TGGGGAAATG CAGTCACTTT TCTTTGGATG CTACACATCC TTTCTGGAAT ATGTCTCACA 1680

CACATCTCTC TTTATCACCC CCTTTTTCAA GTAAACCAAC TTCTTGCAGA AGCTGACAAT 1740

GTGTCTCTTT ACTCTCCACG AAGATTCTGG CCCTTCTCTT CACCTGTCAG AAGTTTAGGA 1800

TTCCAAAGGG ATCATTAGCA TCCATCCCAA CAGCCTGCAC TGCATCCTGA GAACTGCGGT 1860

TCTTGGATCA TCAGGCAACT TTCAACTACA CAGACCAAGG GAGAGAGGGG ACCCCTCCGA 1920

GGTCCCATAG GGTTCTCTGA CATAGTGATG ACCTTTTTCC AAACTTTGAG CAGGGCGCTG 1980

GGGGCCAGGC GTGCGGGAGG GAGGACAAGA ACTCGGGAGT GGCCGAGGAT AAAGCGGGGG 2040

CTCCCTCCAC CCCACGGTGC CCAGTTTCTC CCCGCTGCAC GTGGTCCAGG GTGGTCGCAT 2100

CACCTCTAAA GCCGGTCCCG CCAACCGCCA GCCCCGGGAC TGAACTTGCC CCTCCGGCCG 2160

CCCGCTCCCC GCAGGGGACA GGGGCGGGGA GGGAGAGATC CAGAGGGGGG CTGGGGGAGG 2220

TGGGGCCGCC GGGGAGGAGG CGAGGGAAAC GGGGAGCTCC AGGGAGACGG CTTCCGAGGG 2280

AGAGTGAGAG GGGAGGGCAG CCCGGGCTCG GCACGCTCCC TCCCTCGGCC GCTTTCTCTC 2340

ACATAAGCGC AGGCAGAGGG CGCGTCAGTC ATGCCCTGCC CCTGCGCCCG CCGCCGCCGC 2400

CGCCGCCGCT CAGCCCGGCG CGCTCTGGAG GATCCTGCGC CGCGGCGCTC CCGGGCCCCG 2460

CCGCCGCCAG CCGCCCCGGC GGCCCTCCTC CCGCCCCCGG CACCGCCGCC AGCGCCCCCG 2520

CCGCAGCGCC CGCGGCCCGG CTCCTCTCAC TTCGGGGAAG GGGAGGGAGG AGGGGGACGA 2580

GGGCTCTGGC GGGTTTGGAG GGGCTGAACA TCGCGGGGTG TTCTGGTGTC CCCCGCCCCG 2640

CCTCTCCAAA AAGCTACACC GACGCGGACC GCGGCGGCGT CCTCCCTCGC CCTCGCTTCA 2700

CCTCGCGGGC TCCGAATGCG GGGAGCTCGG ATGTCCGGTT TCCTGTGAGG CTTTTACCTG 2760

ACACCCGCCG CCTTTCCCCG GCACTGGCTG GGAGGGCGCC CTGCAAAGTT GGGAACGCGG 2820

AGCCCCGGAC CCGCTCCCGC CGCCTCCGGC TCGCCCAGGG GGGGTCGCCG GGAGGAGCCC 2880

GGGGGAGAGG GACCAGGAGG GGCCCGCGGC CTCGCAGGGG CGCCCGCGCC CCCACCCCTG 2940

CCCCCGCCAG CGGACCGGTC CCCCACCCCC GGTCCTTCCA CCATGCACTT G 2991

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CACGGCTTAT GCAAGCAAAG 20

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

AACACAGTTT TCCATAATAG 20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Leu Ser Lys Thr Val Ser Gly Ser Glu Gln Asp Leu Pro His Glu Leu
1 5 10 15

His Val Glu

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GACGGACACA GATGGAGGTT TAAAG 25

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

```
Met Arg Thr Leu Ala Cys Leu Leu Leu Leu Gly Cys Gly Tyr Leu Ala
1               5                  10                  15

His Val Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Val Ile Glu Arg
            20                  25                  30

Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
        35                  40                  45

Glu Ile Asp Ser Val Gly Ser Glu Asp Ser Leu Asp Thr Ser Leu Arg
    50                  55                  60

Ala His Gly Val His Ala Thr Lys His Val Pro Glu Lys Arg Pro Leu
65                  70                  75                  80

Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Val Pro Ala Val Cys
                85                  90                  95

Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
            100                 105                 110

Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
        115                 120                 125

Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
    130                 135                 140

Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
145                 150                 155                 160

Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
                165                 170                 175

Cys Ala Cys Ala Thr Thr Ser Leu Asn Pro Asp Tyr Arg Glu Glu Asp
            180                 185                 190

Thr Asp Val Arg
        195
```

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg
1 5 10 15

Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met
20 25 30

Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu
35 40 45

His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met
50 55 60

Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg
65 70 75 80

Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu
85 90 95

Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp
100 105 110

Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln
115 120 125

Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr
130 135 140

Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg
145 150 155 160

Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu
165 170 175

Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr Arg Ser
180 185 190

Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val
195 200 205

Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg
210 215 220

Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly
225 230 235 240

Ala

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 149 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1               5                  10                  15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
            20                  25                  30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
        35                  40                  45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
    50                  55                  60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65                  70                  75                  80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
                85                  90                  95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
            100                 105                 110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
        115                 120                 125

Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Cys Gly Asp
    130                 135                 140

Ala Val Pro Arg Arg
145
```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 191 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15

Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30

Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45

Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80

Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95

Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110

Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125

Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
130 135 140

Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145 150 155 160

Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
165 170 175

Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180 185 190

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 188 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
1 5 10 15

Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
20 25 30

```
Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
        35                  40                  45

Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
    50                  55                  60

Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
65                  70                  75                  80

Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
                85                  90                  95

Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
            100                 105                 110

Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
        115                 120                 125

Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg
    130                 135                 140

Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg
145                 150                 155                 160

Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu
                165                 170                 175

Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
            180                 185
```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 419 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: other
(B) LOCATION: 156
(D) OTHER INFORMATION: /note= "codon 156 can be anything other than cysteine, or can be nothing"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
1               5                   10                  15
```

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe
20 25 30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
35 40 45

Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
50 55 60

Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
65 70 75 80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
85 90 95

Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
100 105 110

His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
115 120 125

Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
130 135 140

Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Xaa Val Ser Val Tyr
145 150 155 160

Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
165 170 175

Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
180 185 190

Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
195 200 205

Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
210 215 220

Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
225 230 235 240

Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
245 250 255

Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
260 265 270

Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
275 280 285

Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
290 295 300

Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
305 310 315 320

```
Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
                325                 330                 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
            340                 345                 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
        355                 360                 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
    370                 375                 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385                 390                 395                 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
                405                 410                 415

Gln Met Ser
```

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 160 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
1               5                   10                  15

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Thr
            20                  25                  30

Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu
        35                  40                  45

Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu
    50                  55                  60

Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe
65                  70                  75                  80

Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn
                85                  90                  95

Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys
            100                 105                 110
```

```
Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val
        115                 120                 125

Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu
    130                 135                 140

Asp Val Tyr Arg Gln Val His Ser Ile Ile His His His His His His
145                 150                 155                 160
```

CLAIMS

1. A purified and isolated polypeptide selected from the group consisting of:

(a) a purified and isolated polypeptide capable of binding to at least one of KDR receptor tyrosine kinase (VEGFR-2) and Flt4 receptor tyrosine kinase (VEGFR-3), said polypeptide comprising a portion of the amino acid sequence in SEQ ID NO: 8 effective to permit such binding;

(b) a purified and isolated VEGF-C of vertebrate origin, wherein said VEGF-C has a molecular weight of about 21-23 kD or about 30-32 kD, as assessed by SDS-PAGE under reducing conditions, and wherein said VEGF-C is capable of binding to Flt4 receptor tyrosine kinase (VEGFR-3);

(c) a purified polypeptide analog of human VEGF-C that is capable of binding to at least one of Flt-1 receptor tyrosine kinase (VEGFR-1), KDR receptor tyrosine kinase (VEGFR-2), and Flt4 receptor tyrosine kinase (VEGFR-3); and

(d) a polypeptide analog of human VEGF that is capable of binding to at least one of VEGFR-1, VEGFR-2, and VEGFR-3, wherein a cysteine residue is introduced in the VEGF amino acid sequence at a position selected from residues 53 to 63 of the human VEGF165 precursor having the amino acid sequence set forth in SEQ ID NO: 56.

2. A purified and isolated polypeptide according to claim 1 that is capable of binding to at least one of KDR receptor tyrosine kinase (VEGFR-2) and Flt4 receptor tyrosine kinase (VEGFR-3), said polypeptide comprising a portion of the amino acid sequence in SEQ ID NO: 8 effective to permit such binding.

3. A polypeptide according to claim 1 or 2, wherein said polypeptide is capable of stimulating tyrosine phosphorylation of a receptor selected from the group consisting of VEGFR-2 and VEGFR-3 in a host cell expressing said receptor.

4. A purified and isolated polypeptide multimer, wherein at least one monomer thereof is a polypeptide according to any one of claims 1-3, and wherein said multimer is capable of binding to at least one of VEGFR-2 and VEGFR-3.

5. A multimer according to claim 4 having a VEGF-C biological activity.

6. A multimer according to claim 4 or 5 wherein at least one monomer thereof is selected from the group consisting of a vascular endothelial growth factor (VEGF) polypeptide, a vascular endothelial growth factor B (VEGF-B) polypeptide, a platelet derived growth factor A (PDGF-A) polypeptide, a platelet derived growth factor B (PDGF-B) polypeptide, a *c-fos* induced growth factor (FIGF) polypeptide, and a placenta growth factor (PlGF) polypeptide.

7. A dimer according to claim 4, 5, or 6.

8. A dimer according to claim 7 wherein each monomer thereof is capable of binding to at least one of VEGFR-2 and VEGFR-3 and has an amino acid sequence comprising a portion of SEQ ID NO: 8 effective to permit such binding.

9. A dimer according to claim 7 or 8 wherein the two monomers are free of covalent attachments to each other.

10. A purified and isolated polypeptide according to claim 1 that is a VEGF-C of vertebrate origin, wherein said VEGF-C has a molecular weight of about 21-23 kD, as assessed by SDS-PAGE under reducing conditions, and wherein said VEGF-C is capable of binding to Flt4 receptor tyrosine kinase (VEGFR-3).

11. A purified and isolated polypeptide according to claim 1 that is a VEGF-C of vertebrate origin, wherein said VEGF-C has a molecular weight of about 30-32 kD, as assessed by SDS-PAGE under reducing conditions, and wherein said VEGF-C is capable of binding to Flt4 receptor tyrosine kinase (VEGFR-3).

12. A polypeptide analog of the VEGF-C according to claim 10 or 11, wherein a conserved cysteine residue in said VEGF-C has been deleted or replaced, and wherein said analog is capable of binding to VEGFR-3 and has reduced VEGFR-2 binding affinity relative to said VEGF-C.

13. A polypeptide analog according to claim 10 or 11 wherein said conserved cysteine residue corresponds to the cysteine at position 156 of SEQ ID NO: 8.

14. A purified polypeptide according to claim 1 that is an analog of human VEGF-C that is capable of binding to at least one of Flt-1 receptor tyrosine kinase (VEGFR-1), KDR receptor tyrosine kinase (VEGFR-2), and Flt4 receptor tyrosine kinase (VEGFR-3).

15. A polypeptide according to claim 14 that binds VEGFR-3 and has reduced VEGFR-2 binding affinity relative to human VEGF-C having an amino acid sequence consisting essentially of amino acids 103-227 of SEQ ID NO: 8.

16. A polypeptide according to claim 14 or 15 that is a VEGF-C ΔC_{156} polypeptide.

17. A VEGF-C ΔC_{156} polypeptide according to claim 16 comprising amino acids 131 to 211 of SEQ ID NO: 8, wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been deleted or replaced.

18. A VEGF-C ΔC_{156} polypeptide according to claim 16 or 17 comprising a continuous portion of SEQ ID NO: 8, said portion having as its amino terminal residue an amino acid between residues 102 and 114 of SEQ ID NO: 8, and having as its carboxy terminal residue an amino acid between residues 212 and 228 of SEQ ID NO: 8, wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been deleted or replaced.

19. A VEGF-C ΔC_{156} polypeptide according to any one of claims 16-18 wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been replaced by a serine residue.

20. A polypeptide according to claim 14 or 15 that is a VEGF-C $\Delta R_{226}\Delta R_{227}$ polypeptide.

21. A VEGF-C $\Delta R_{226}\Delta R_{227}$ polypeptide according to claim 20 having an amino acid sequence comprising amino acids 112-419 of SEQ ID NO: 8, wherein the arginine residues at positions 226 and 227 of SEQ ID NO: 8 have been deleted or replaced.

22. A polypeptide according to claim 14 that is a human VEGF-C^{basic} polypeptide.

23. A polypeptide according to claim 22 having an amino acid sequence comprising residues 131 to 211 of SEQ ID NO: 8, wherein the glutamic acid residue at position 187, the threonine residue at position 189, and the proline residue at position 191 of SEQ ID NO: 8 have been replaced by an arginine residue, a lysine residue, and a histidine residue, respectively.

24. A composition comprising a polypeptide according to any one of claims 1-4 and 10-23, and further comprising a purified myelopoietic growth factor in admixture therewith.

25. A kit useful for modulating myelopoiesis comprising: a first composition comprising a polypeptide according to any one of claims 1-4 and 10-23, packaged with at least one additional composition comprising a myelopoietic growth factor.

26. A composition according to claim 24 or a kit according to claim 25 wherein the myelopoietic growth factor is selected from the group consisting of granulocyte colony stimulating factor (G-CSF), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), interleukin-3 (IL-3), stem cell factor (SCF), and combinations thereof.

27. A composition comprising a polypeptide according to any one of claims 1-4 and 10-23, and further comprising a purified polypeptide selected from the group consisting of vascular endothelial growth factor (VEGF) polypeptides, vascular endothelial growth factor B (VEGF-B) polypeptides, platelet derived growth factor A (PDGF-A) polypeptides, platelet derived growth factor B (PDGF-B) polypeptides, *c-fos* induced growth factor (FIGF) polypeptides, and placenta growth factor (PlGF) polypeptides.

28. A polypeptide according to claim 1 that is an analog of human VEGF, wherein a cysteine residue is introduced in the VEGF amino acid sequence at a position selected from residues 53 to 63 of the human VEGF165 precursor having the amino acid sequence set forth in SEQ ID NO: 56, and wherein the polypeptide is capable of binding to at lest one of VEGFR-1, VEGFR-2, and VEGFR-3.

29. An analog according to claim 28 wherein said cysteine is introduced at position 58 of the VEGF165 precursor having the amino acid sequence set forth in SEQ ID NO: 56.

30. A purified and isolated nucleic acid comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1-4, 10-23, and 28-29.

31. A vector comprising a nucleic acid according to claim 30.

32. A host cell transformed or transfected with a nucleic acid according to claim 30 or a vector according to claim 31.

33. A method of making a polypeptide capable of specifically binding to at least one of VEGFR-1, VEGFR-2, and VEGFR-3, said method comprising the steps of:

(a) expressing a nucleic acid according to claim 30 or a vector according to claim 31 in a host cell; and

(b) purifying a polypeptide capable of specifically binding to at least one of VEGFR-1, VEGFR-2, and VEGFR-3 from said host cell or from a growth medium of said host cell.

34. An antibody which is specifically reactive with a polypeptide according to any one of claims 1-4, 10-23, and 28-29.

35. A pharmaceutical composition comprising an antibody according to claim 34 in a pharmaceutically-acceptable diluent, adjuvant, excipient, or carrier.

36. A pharmaceutical composition comprising a polypeptide according to any one of claims 1-4, 10-23, and 28-29 in a pharmaceutically-acceptable diluent, adjuvant, excipient, or carrier.

37. A method of modulating the proliferation of mammalian endothelial cells comprising the step of contacting mammalian endothelial cells with a polypeptide in an amount effective to modulate the proliferation of mammalian endothelial cells, said polypeptide selected from the group consisting of:

(a) a polypeptide according to any one of claims 1-4, 10-23, and 28-29; and

(b) a polypeptide comprising an antigen binding portion of an anti-VEGF-C antibody.

38. A method of increasing the proliferation of mammalian endothelial cells according to claim 37, comprising contacting mammalian endothelial cells with a polypeptide in an amount effective to increase the proliferation of mammalian endothelial cells.

39. A method according to claim 37 or 38 wherein said endothelial cells are lymphatic endothelial cells.

40. An *in vivo* method according to claim 39 wherein the contacting step comprises administering to a mammalian subject in need of modulation of the proliferation of lymphatic endothelial cells a composition comprising said polypeptide, in an amount effective to modulate the proliferation of lymphatic endothelial cells *in vivo*.

41. A method according to claim 40 wherein said polypeptide has reduced effect on the permeability of mammalian blood vessels compared to a wildtype VEGF-C polypeptide with an amino acid sequence set forth in SEQ ID NO: 8 from residue 103 to residue 227.

42. A method according to any one of claims 37- 41 wherein said polypeptide is a VEGF-C ΔC_{156} polypeptide.

43. A method for modulating myelopoiesis in a mammalian subject comprising administering to a mammalian subject in need of modulation of myelopoiesis an amount of a polypeptide effective to modulate myelopoiesis, said polypeptide selected from the group consisting of:

(a) a polypeptide according to any one of claims 1-4, 10-23, and 28-29; and

(b) a polypeptide comprising an antigen binding portion of an anti-VEGF-C antibody.

44. A method according to claim 43 wherein said mammalian subject suffers from granulocytopenia, and said method comprises administering to said subject an amount of a polypeptide effective to stimulate myelopoiesis.

45. A method according to claim 43 or 44 comprising administering to said subject an amount of a polypeptide effective to increase the neutrophil count in blood of said subject.

46. A method according to any one of claims 43-45 wherein said mammalian subject is human.

47. A method according to any one of claims 43-46 further comprising administering to said subject a myelopoietic growth factor selected from the group consisting of granulocyte colony stimulating factor (G-CSF), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), interleukin-3 (IL-3), stem cell factor (SCF), and combinations thereof.

48. A method of modulating the proliferation of neutrophilic granulocytes in vitro comprising the step of contacting mammalian stem cells with a polypeptide in an amount effective to modulate the proliferation of neutrophilic granulocytes, said polypeptide selected from the group consisting of:

(a) a polypeptide according to any one of claims 1-4, 10-23, and 28-29; and

(b) a polypeptide comprising an antigen binding portion of an anti-VEGF-C antibody.

49. A method of modulating the proliferation and/or differentiation of mammalian CD34+ progenitor cells comprising contacting mammalian CD34+ progenitor cells with a polypeptide according to any one of claims 1-4, 10-23, and 28-29, in an amount effective to modulate the proliferation and/or differentiation of the cells.

50. A method according to claim 49 further comprising contacting the mammalian CD34+ progenitor cells with a myelopoietic growth factor selected from the group consisting of granulocyte colony stimulating factor (G-CSF), macrophage-CSF (M-CSF), granulocyte-macrophage-CSF (GM-CSF), interleukin-3 (IL-3), stem cell factor (SCF), and combinations thereof, in an amounts effective to modulate the proliferation of CD34+ progenitor cells when used in combination with said polypeptide.

51. A method according to claim 49 or 50 wherein the contacting is performed *in vitro* by culturing mammalian CD34+ progenitor cells in the presence of the polypeptide and optionally the myelopoietic growth factor.

52. A method of increasing the number of neutrophils in the blood of a mammalian subject comprising the step of expressing in a cell in a subject in need of an increased number of blood neutrophils a DNA encoding a VEGF-C protein, said DNA operatively linked to a non-VEGF-C promoter or other non-VEGF-C control sequence that promotes expression of said DNA in said cell.

53. A method of increasing the number of endothelial cells in a mammalian subject comprising the step of expressing in a cell in a subject in need of an increased number of endothelial cells a DNA encoding a VEGF-C protein, said DNA operatively linked to a non-VEGF-C promoter or other non-VEGF-C control sequence that promotes expression of said DNA in said cell.

54. A cell comprising a nucleic acid having a sequence encoding human VEGF-C and further comprising a non-VEGF-C promoter sequence or other non-VEGF-C control sequence that increases RNA transcription in said cell of said sequence encoding human VEGF-C.

55. A purified nucleic acid comprising a VEGF-C promoter sequence.

56. A nucleic acid according to claim 55 comprising a portion of SEQ ID NO: 48, wherein said portion is capable of promoting expression of a protein encoding gene operatively linked thereto under conditions wherein VEGF-C is expressed in native host cells.

57. A chimeric nucleic acid comprising a nucleic acid according to claim 55 or 56 operatively connected to a sequence encoding a protein other than a human VEGF-C.

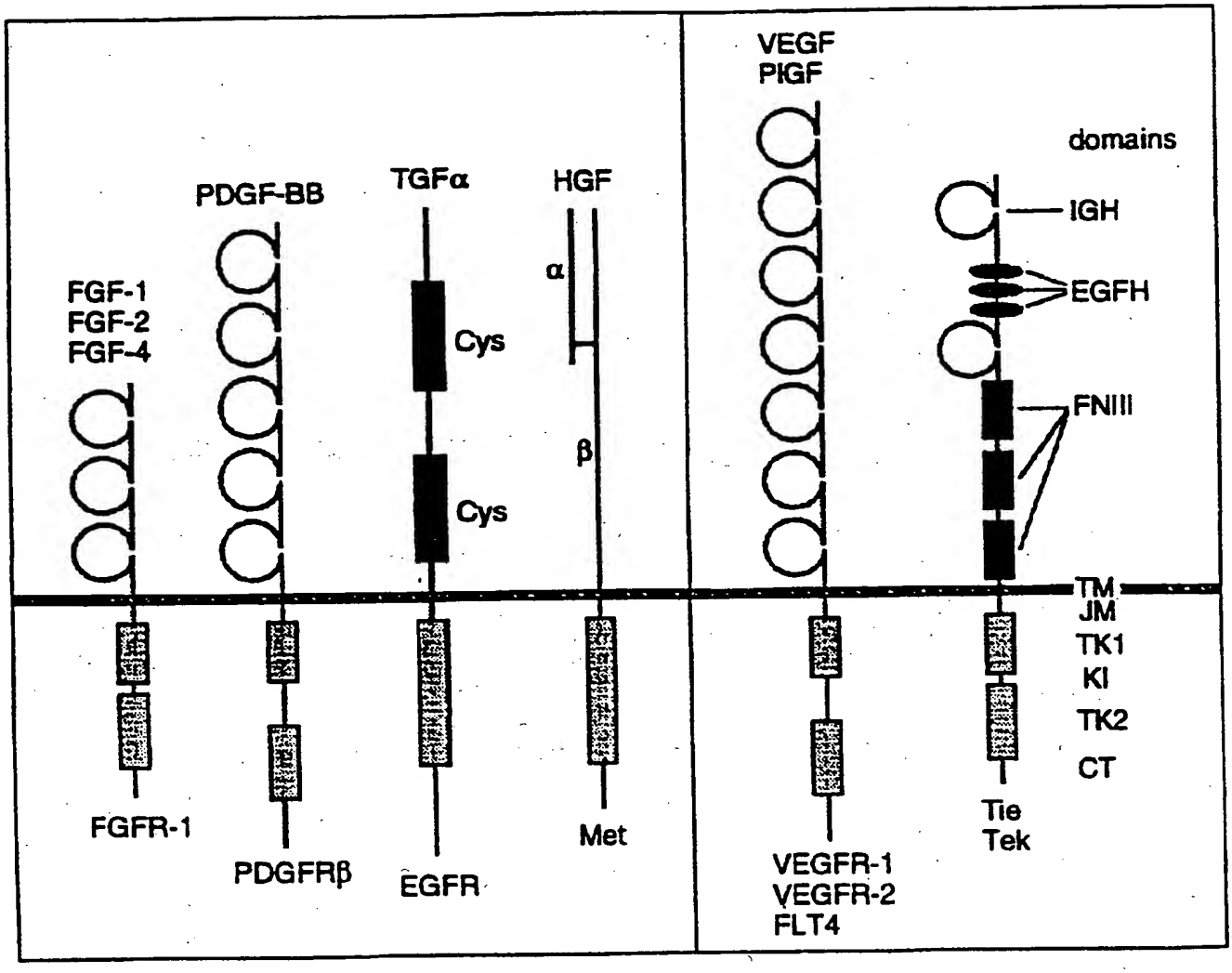
VEGF
PlGF
domains
PDGF-BB
TGFα
HGF
IGH
α
FGF-1
FGF-2
FGF-4
Cys
EGFH
β
FNIII
Cys
TM
JM
TK1
KI
TK2
CT
FGFR-1
PDGFRβ
EGFR
Met
Tie
Tek
VEGFR-1
VEGFR-2
FLT4

FIGURE 1

```
            1                                                  50
   PDGF-A   .......... .......... .......... .......... .MRTLACLLL
   PDGF-B   .......... .......... .......... .......... MNRCWA.LFL
   PlGF-1   .......... .......... .......... .......... ..........
 VEGF165    .......... .......... .......... .......... ..........
VEGF-B167   .......... .......... .......... .......... ..........
   VEGF-C   MHLLGFFSVA CSLLAAALLP GPREAPAAAA AFESGLDLSD AEPDAGEATA

            51                                                 100
   PDGF-A   LGCGYLAHVL AEEAEIPREV IERLARSQIH SIRDLQRLLE IDSVGSEDSL
   PDGF-B   SLCCYLRLVS AEGDPIPEEL YEMLSDHSIR SFDDLQRLLH GDP.GEEDGA
   PlGF-1   .......... ......MPVM RLFPC..FLQ LLAGLAL... PAVPPQQW..
 VEGF165    .......... .........M NFLLS..WVH WSLALLLYLH HAKWSQAA..
VEGF-B167   .......... .........M SPLLR..RLL LAALLQLAPA QAPVSQP...
   VEGF-C   YASKDLEEQL RSVSSVDELM TVLYPEYWKM YKCQLRKGGW QHNREQANLN

            101                                                150
   PDGF-A   DTSLRAHGVH ATKHVPEKRP LPIRRKRSI. .....EEAVP AVCKTRTVIY
   PDGF-B   ELDLNMTRSH SGGELES... .LARGRRSLG SLTIAEPAMI AECKTRTEVF
   PlGF-1   .....ALSAG NGSSEVEVVP FQE.VWGR.. .......... SYCRALERLV
 VEGF165    .....PMAEG GGQNHHEVVK FMD.VYQR.. .......... SYCHPIETLV
VEGF-B167   .........D APGHQRKVVS WID.VYTR.. .......... ATCQPREVVV
   VEGF-C   SRTEETIKFA AAHYNTEILK SIDNEWRK.. .......... TQCMPREVCI

            151                                                200
   PDGF-A   EIPRSQVDPT SANFLIWPPC VEVKRCTGCC NTSSVKCQPS RVHHRSVKVA
   PDGF-B   EISRRLIDRT NANFLVWPPC VEVQRCSGCC NNRNVQCRPT QVQLRPVQVR
   PlGF-1   DVVSEYPSEV ..EHMFSPSC VSLLRCTGCC GDENLHCVPV ETANVTMQLL
 VEGF165    DIFQEYPDEI ..EYIFKPSC VPLMRCGGCC NDEGLECVPT EESNITMQIM
VEGF-B167   PLTVELMGTV ..AKQLVPSC VTVQRCGGCC PDDGLECVPT GQHQVRMQIL
   VEGF-C   DVGKEFGVAT ..NTFFKPPC VSVYRCGGCC NSEGLQCMNT STSYLSKTLF
```

FIGURE 2 A

```
      201                                                        250
  PDGF-A  KVEYVRKKPK LKEVQVRLEE HLECACAT.. .......... TSLNPDYREE
  PDGF-B  KIEIVRKKPI FKKATVTLED HLACKCETVA AARPVTRSPG GSQEQRAKTP
  PlGF-1  KIRSG..DRP .SYVELTFSQ HVRCECRPLR EK........ ..........
VEGF165   RIKPH..QGQ .HIGEMSFLQ HNKCECRPKK DR........ ..........
VEGF-B167 MIRYP..SSQ ..LGEMSLEE HSQCECRPKK KD........ ..........
  VEGF-C  EITVPLSQGP .KPVTISFAN HTSCRCMSKL DVYRQVHSII RRSLPATLPQ

          251                                                    300
  PDGF-A  DTDVR..... .......... .......... .......... ..........
  PDGF-B  QTRVTIRTVR VRRPPKGKHR KFKHTHDKTA LKETLGA... ..........
  PlGF-1  .......... .......... .......... .MKPERCGDA VPRR......
VEGF165   .......... .......... .......... .ARQENPCGP CSERRKHLFV
VEGF-B167 .......... .......... .........S AVKPDSPRPL CPRCTQHHQR
  VEGF-C  CQAANKTCPT NYMWNNHICR CLAQEDFMFS SDAGDDSTDG FHDICGPNKE

          301                                                    350
  PDGF-A  .......... .......... .......... .......... ..........
  PDGF-B  .......... .......... .......... .......... ..........
  PlGF-1  .......... .......... .......... .......... ..........
VEGF165   QDPQTCKCSC KNTDS.RCKA RQLELNERTC RCDKPRR... ..........
VEGF-B167 PDPRTCRCRC RRRSFLRCQG RGLELNPDTC RCRKLRR... ..........
  VEGF-C  LDEETCQCVC RAGLRPASCG PHKELDRNSC QCVCKNKLFP SQCGANREFD
```

FIGURE 2 B

```
             351                                                    400
PDGF-A       .......... .......... .......... .......... ..........
PDGF-B       .......... .......... .......... .......... ..........
PlGF-1       .......... .......... .......... .......... ..........
VEGF165      .......... .......... .......... .......... ..........
VEGF-B167    .......... .......... .......... .......... ..........
VEGF-C       ENTCQCVCKR TCPRNQPLNP GKCACECTES PQKCLLKGKK FHHQTCSCYR

             401                              434
PDGF-A       .......... .......... .......... ....
PDGF-B       .......... .......... .......... ....
PlGF-1       .......... .......... .......... ....
VEGF165      .......... .......... .......... ....
VEGF-B167    .......... .......... .......... ....
VEGF-C       RPCTNRQKAC EPGFSYSEEV CRCVPSYWKR PQMS
```

FIGURE 2 C

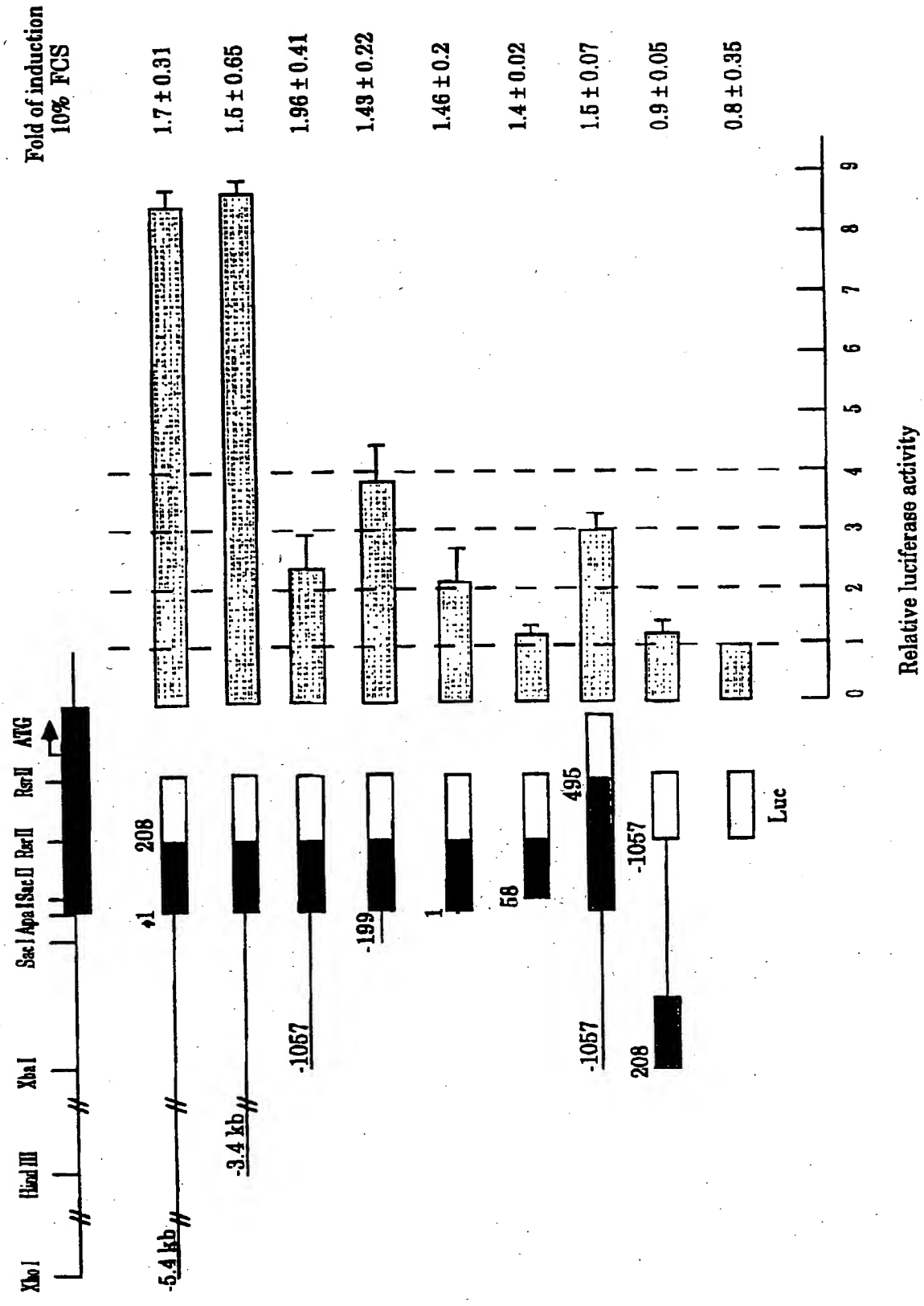
Fold of induction
10% FCS
1.7 ± 0.31
1.5 ± 0.65
1.96 ± 0.41
1.43 ± 0.22
1.46 ± 0.2
1.4 ± 0.02
1.5 ± 0.07
0.9 ± 0.05
0.8 ± 0.35
Xba I
Hind III
Xba I
Sac I Apa I Sac II Rsa II
Rsa II
ATG
-5.4 kb
-3.4 kb
-1057
-199
1
58
495
-1057
208
-1057
208
Luc
0 1 2 3 4 5 6 7 8 9
Relative luciferase activity

FIGURE 3

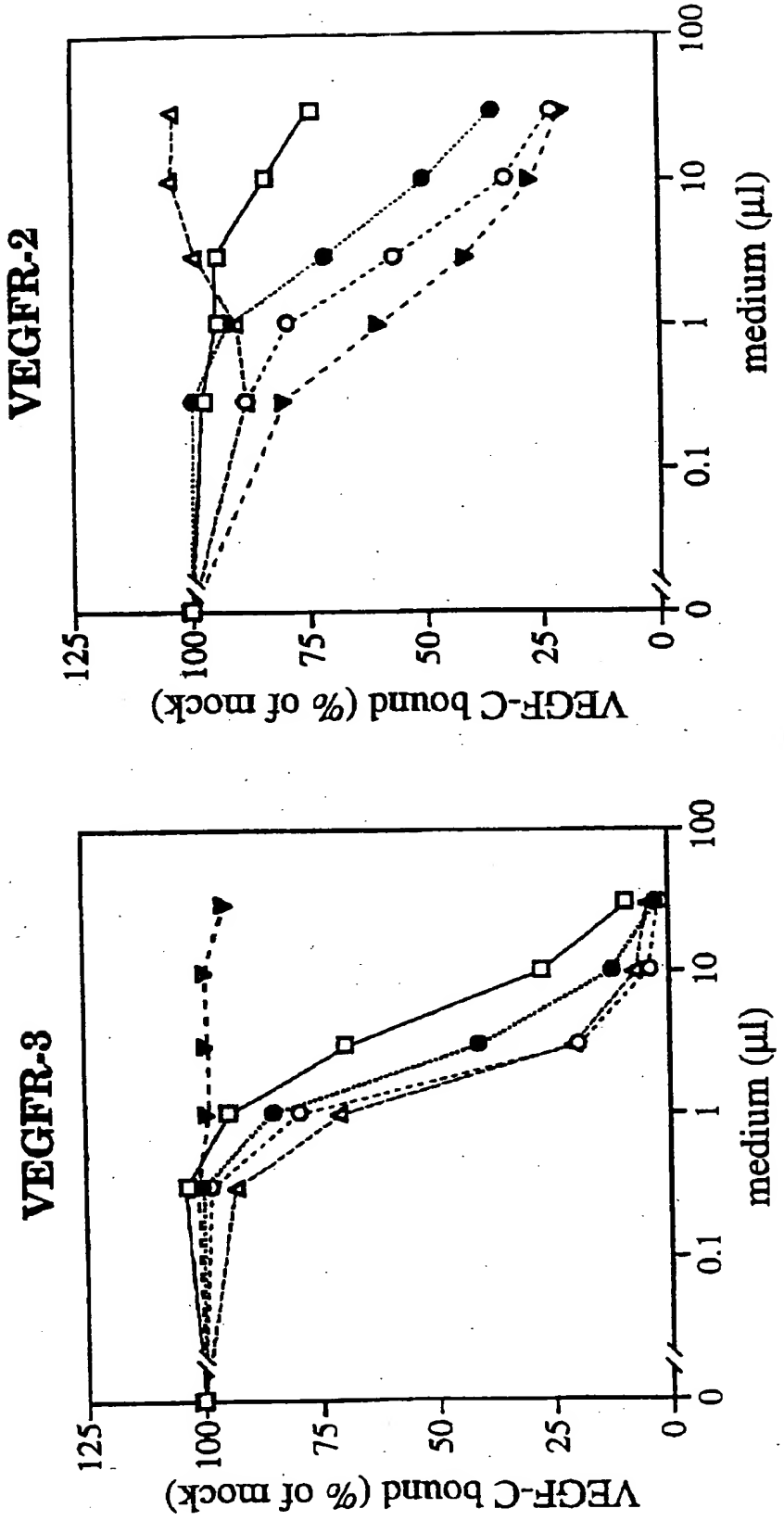
VEGFR-2
VEGFR-3
VEGF-C bound (% of mock)
125
100
75
50
25
0
0
0.1
1
10
100
medium (µl)

FIGURE 4

VEGF-C alignment

```
      1                                                          50
Hum   HMLLGFFSVA CSLLAAALLP GPREAPAAAA AFESGLDLSD AEPDAGEATA
Mou   MHLLCFLSLA CSLLAAALIP SPREAPATVA AFESGLGFSE AEPDGGEVKA
Qua   MHLLEMLSLG CCLAAGAVLL GPRQPPVA.A AYESGHGYYE EEPGAGEPKA

      51                                                         100
Hum   YASKDLEEQL RSVSSVDELM TVLYPEYWKM YKCQLRKGGW QHNREQANLN
Mou   FEGKDLEEQL RSVSSVDELM SVLYPDYWKM YKCQLRKGGW Q....QPTLN
Qua   HASKDLEEQL RSVSSVDELM TVLYPEYWKM FKCQLRKGGW QHNREHSSSD

      101                                                        150
Hum   SRTEETIKFA AAHYNTEILK SIDNEWRKTQ CMPREVCIDV GKEFGVATNT
Mou   TRTGDSVKFA AAHYNTEILK SIDNEWRKTQ CMPREVCIDV GKEFGAATNT
Qua   TRSDDSLKFA AAHYNAEILK SIDTEWRKTQ GMPREVCVDL GKEFGATTNT

      151                                                        200
Hum   FFKPPCVSVY RCGGCCNSEG LQCMNTSTSY LSKTLFEITV PLSQGPKPVT
Mou   FFKPPCVSVY RCGGCCNSEG LQCMNTSTGY LSKTLFEITV PLSQGPKPVT
Qua   FFKPPCVSIY RCGGCCNSEG LQCMNISTNY ISKTLFEITV PLSHGPKPVT

      201                                                        250
Hum   ISFANHTSCR CMSKLDVYRQ VHSIIRRSLP ATLPQCQAAN KTCPTNYMWN
Mou   ISFANHTSCR CMSKLDVYRQ VHSIIRRSLP ATLPQCQAAN KTCPTNYVWN
Qua   VSFANHTSCR CMSKLDVYRQ VHSIIRRSLP ATQTQCHVAN KTCPKNHVWN

      251                                                        300
Hum   NHICRCLAQE DFMFSSDAGD DSTDGFHDIC GPNKELDEET CQCVCRAGLR
Mou   NYMCRCLAQQ DFIFYSNVED DSTNGFHDVC GPNKELDEDT CQCVCKGGLR
Qua   NQICRCLAQH DFGFSSHLGD SDTSEGFHIC GPNKELDEET CQCVCKGGVR

      301                                                        350
Hum   PASCGPHKEL DRNSCQCVCK NKLFPSQCGA NREFDENTCQ CVCKRTCPRN
Mou   PSSCGPHKEL DRDSCQCVCK NKLFPNSCGA NREFDENTCQ CVCKRTCPRN
Qua   PISCGPHKEL DRASCQCMCK NKLLPSSCGP NKEFDEEKCQ CVCKKTCPKH

      351                                                        400
Hum   QPLNPGKCAC ECTESPQKCL LKGKKFHHQT CSCYRRPCTN RQKACEPGFS
Mou   QPLNPGKCAC ECTENTQKCF LKGKKFHHQT CSCYRRPCAN RLKHCDPGLS
Qua   HPLNPAKCIC ECTESPNKCF LKGKRFHHQT CSCYRPPCTV RTKRCDAGFL

      401                420
Hum   YSEEVCRCVP SYWKRPQMS*
Mou   FSEEVCRCVP SYWKRPHLN.
Qua   LAEEVCRCVR TSWKRPLMN*
```

FIGURE 5

FIGURE 6A

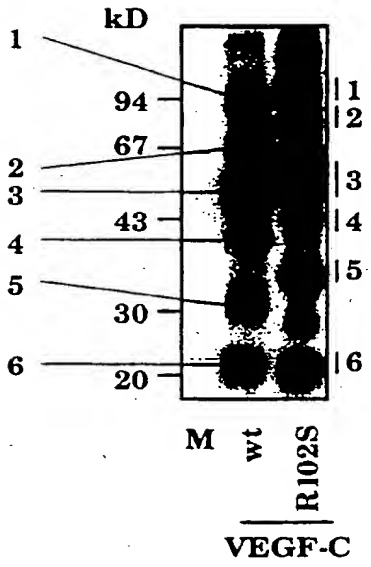

FIGURE 6B

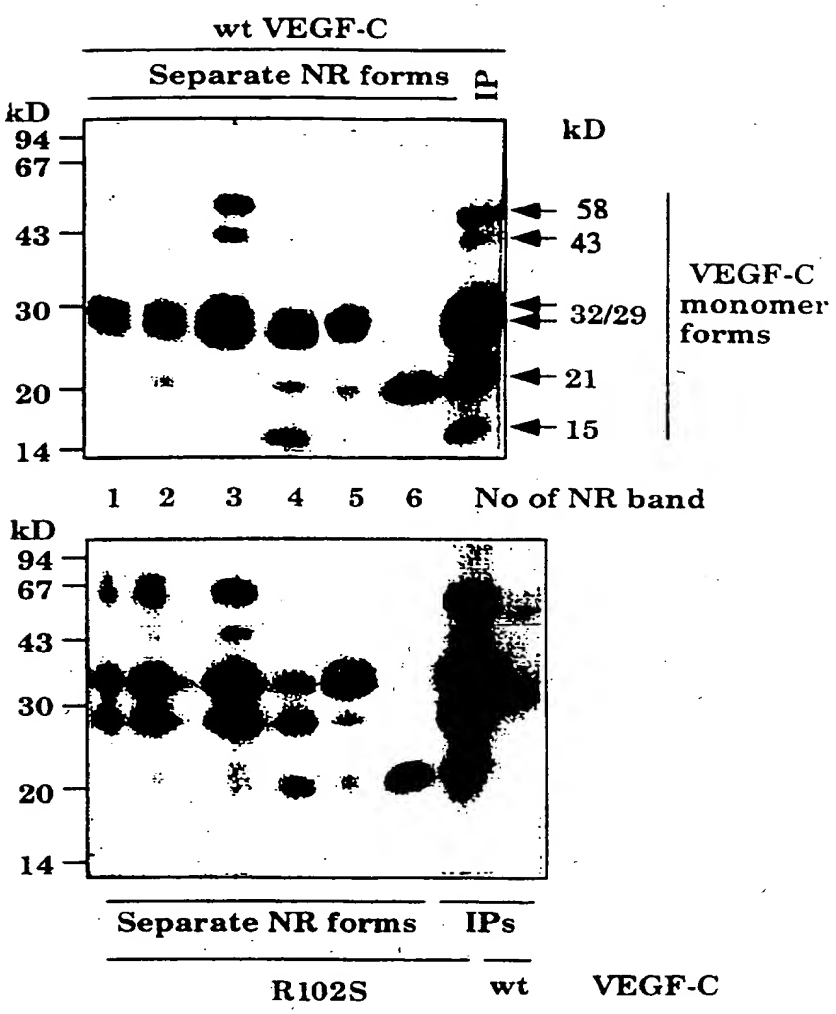

FIGURE 6C

Media

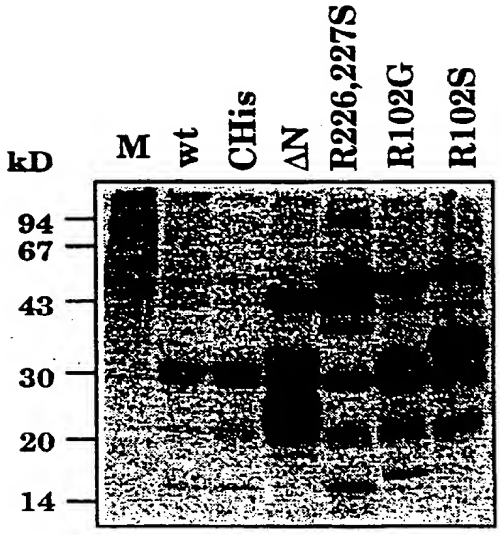

FIGURE 7A

Lysates

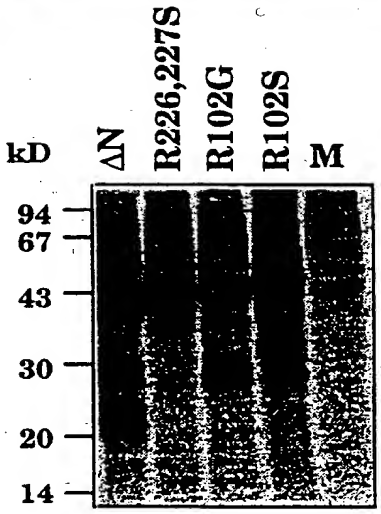

FIGURE 7B

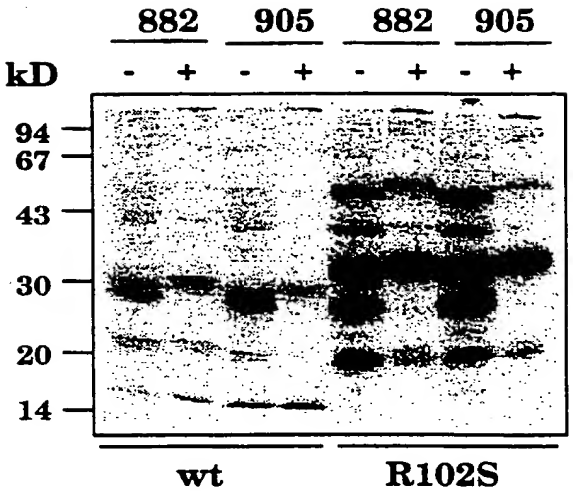

FIGURE 8A

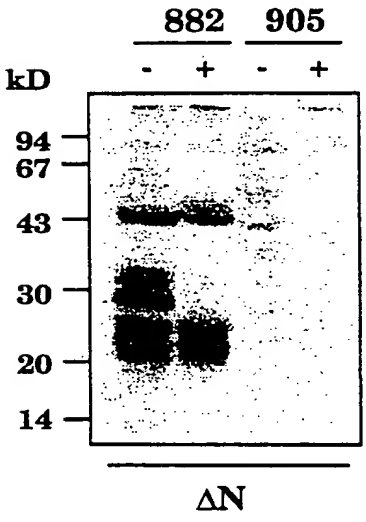

FIGURE 8B

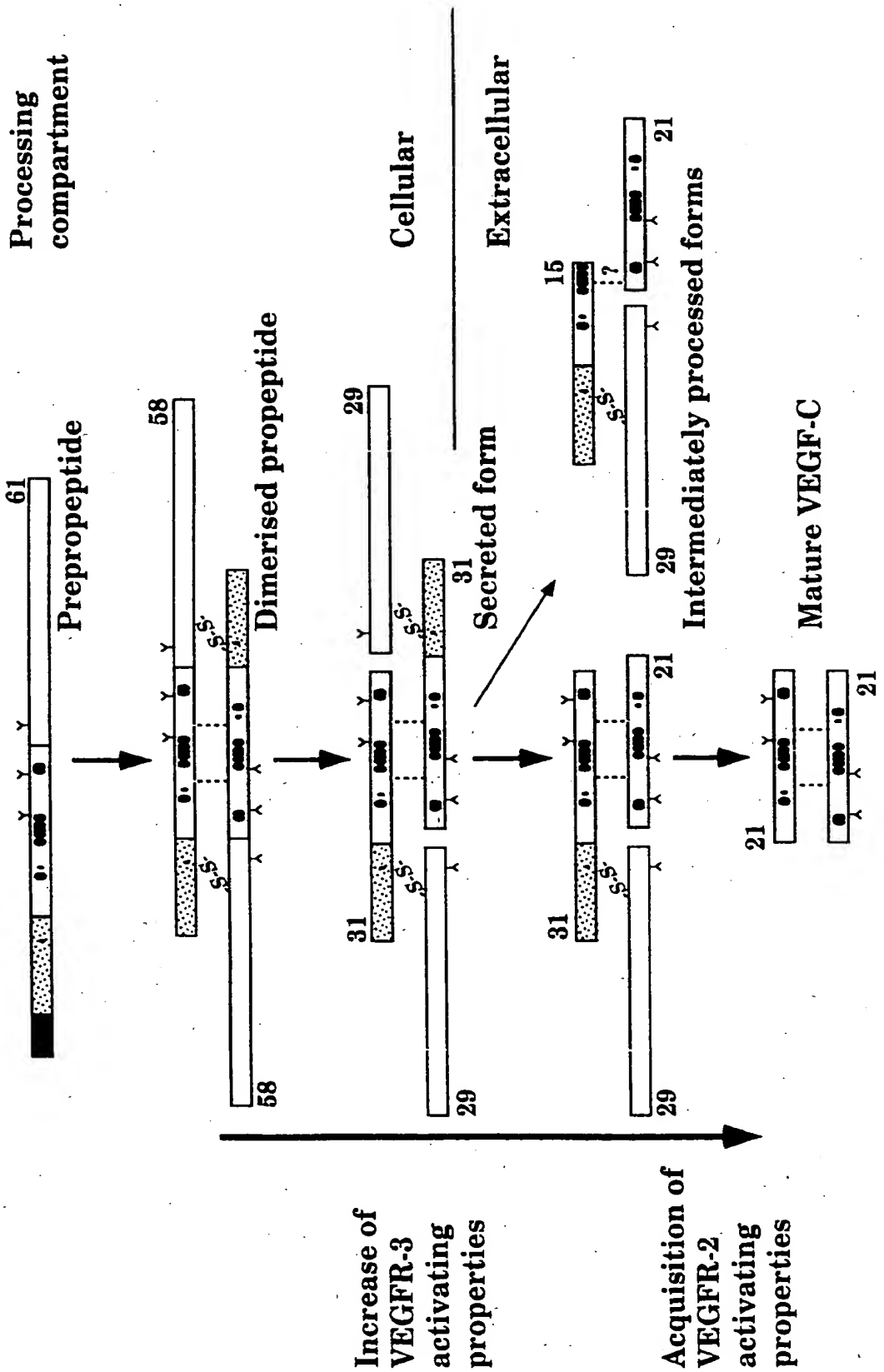
Processing compartment
Cellular
Extracellular
Prepropeptide
Dimerised propeptide
Secreted form
Intermediately processed forms
Mature VEGF-C
Increase of VEGFR-3 activating properties
Acquisition of VEGFR-2 activating properties
61
58
58
29
31
29
31
29
15
?
29
21
31
21
21
21

FIGURE 9

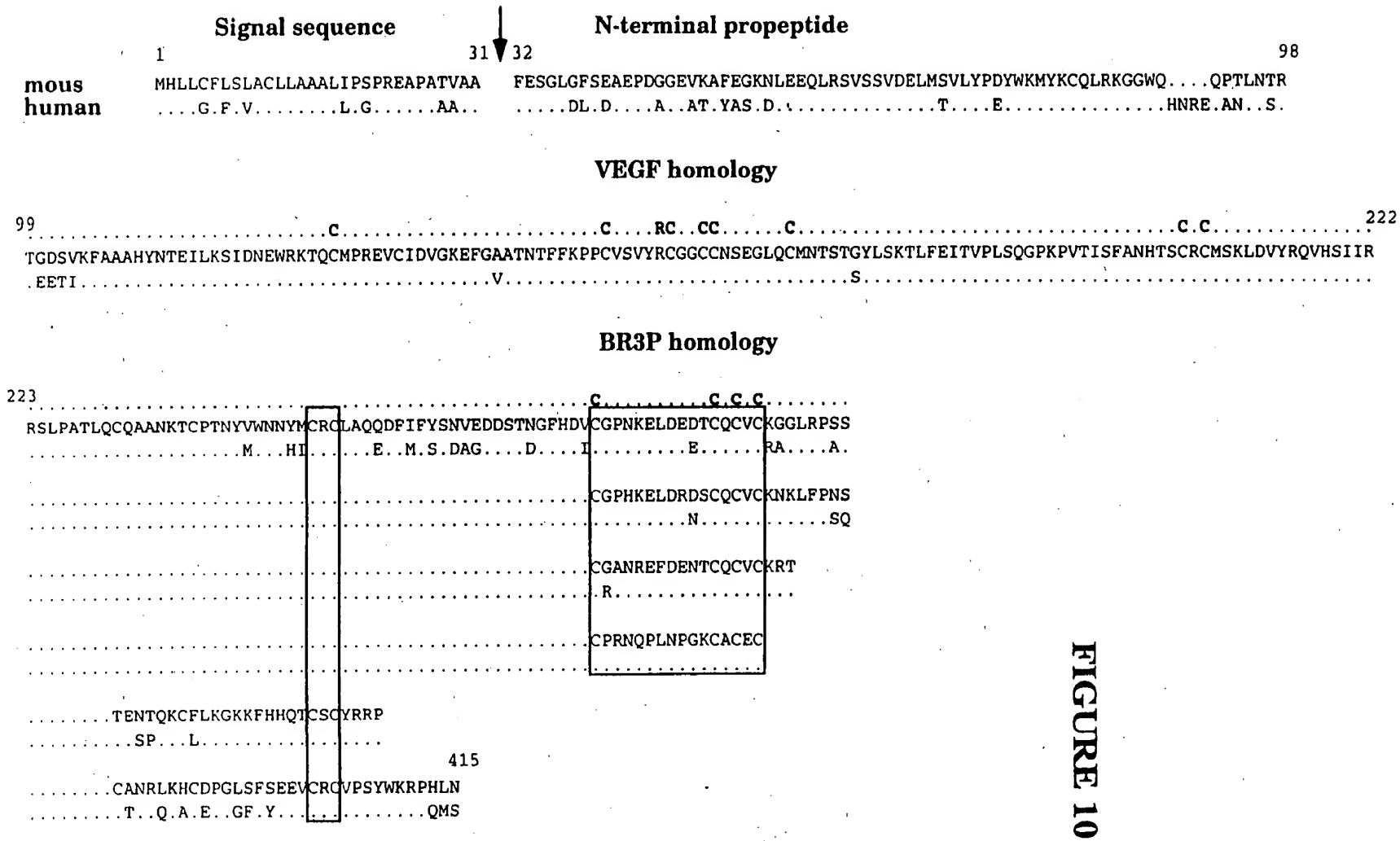
Signal sequence
N-terminal propeptide
1
31
32
98
mous MHLLCFLSLACLLAAALIPSPREAPATVAA FESGLGFSEAEPDGGEVKAFEGKNLEEQLRSVSSVDELMSVLYPDYWKMYKCQLRKGGWQ....QPTLNTR
human ....G.F.V.....L.G.....AA.. .....DL.D....A..AT.YAS.D.....T....E.....HNRE.AN..S.
VEGF homology
99
222
TGDSVKFAAAHYNTEILKSIDNEWRKTQCMPREVCIDVGKEFGAATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTGYLSKTLFEITVPLSQGPKPVTISFANHTSCRCMSKLDVYRQVHSIIR
.EETI
BR3P homology
223
RSLPATLQCQAANKTCPTNYVWNNYMCRCLAQQDFIFYSNVEDDSTNGFHDVCGPNKELDEDTCQCVCKGGLRPSS
CGPHKELDRDSCQCVCKNKLFPNS
CGANREFDENTCQCVCKRT
CPRNQPLNPGKCACEC
TENTQKCFLKGKKFHHQTCSCYRRP
415
CANRLKHCDPGLSFSEEVCRCVPSYWKRPHLN

FIGURE 10

HUMAN

Exon length	**Donor site**	**Intron length**	**Acceptor site**

```
.....G...E...A...T(49).....A...Y...A...S.
E1.....GGC.GAG.GCC.ACG.gtaggtctgcgt...>10.kb..TTTCTTTGACAG.GCT.TAT.GCA.AGC
.....E...I...L...K(116).....S...I...D...N.
E2.214.bp..GAG.ATC.TTG.AAA.Agtaagtatggg...1.6.kb....atgacttgacagGT.ATT.GAT.AAT
.....L...S...K...T(180).....L...F...E...I.
E3.191.bp..CTC.AGC.AAG.ACG.gtgggtattgt.....9.kb.cccttctttgtag.TTA.TTT.GAA.ATT
.....T...L...P...Q(231).....C...Q...A...A.
E4.152.bp..ACA.CTA.CCA.CAgtgagtatgaattaaa.>10.kb..ttcttccaaagG.TGT.CAG.GCA.GCG
.....A...G...D...(266).....D...S...T...D.
E5.107.bp..GCT.GGA.GAT.Ggtagcagaatg.....301.bp....ctatttgtctagAC.TCA.ACA.GAT
.....Q...T...C...S(378).....C...Y...R...R.
E6.334.bp..CAA.ACA.TGC.AGgtaagagatcc.....>10.kb..tgttctcctagC.TGT.TAC.AGA.CGG
.....Q...M...S(419)Stop.....
E7.(501).bp..CAA.ATG.AGC.TAA.GTATGTACTGTT...ATTGTATTAT
```

FIGURE 11A

MOUSE

Exon length	Donor site	Intron length	Acceptor site

```
.............G...E...V...K(49).....................................A...F...E...G.
E1.........GGC.GAG.GTC.AAG.gtaggtgcaagg.>10.kb.attgtctttgacag.GCT.TTT.TGA.AGG
.............E...I...L...K(116).....................................S...I...D...N.
E2.201.bp..GAG.ATC.CTG.AAA.Agtaagtag......4.kb....tgtgactcgacagGT.ATT.GAT.AAT
.............L...S...K...T(180).....................................L...F...E...I.
E3.191.bp..CTC.AGC.AAG.ACG.gtaggtat.......9.kb..ttgtcccttgtag.TTG.TTT.GAA.ATT
.............T...L...P...Q(231).....................................C....Q...A...A.
E4.152.bp..ACA.TTA.CCA.CAgtgagtatg.......10.kb.gtctccccaaaagG.TGT.CAG.GCA.GCT
.............N...V...E...D(266).....................................D....S...T...N.
E5.107.bp..AAT.GTT.GAA.GAT.Ggtaagtaaaa...350.bp..........tctagAC.TCA.ACC.AAT
.............Q...T...C...S(378).....................................C...Y...R...R.
E6.334.bp..CAA.ACA.TGC.AGgtaaggagtgt.......6.kb..ttttcccctagT.TGT.TAC.AGA.AGA
.............H...L...N(415)Stop..............polyA........
E7.506.bp..CAT.CTG.AAC.TAA.GATCATACC...ATTGTATTATAAgctgtgaag
```

FIGURE 11B

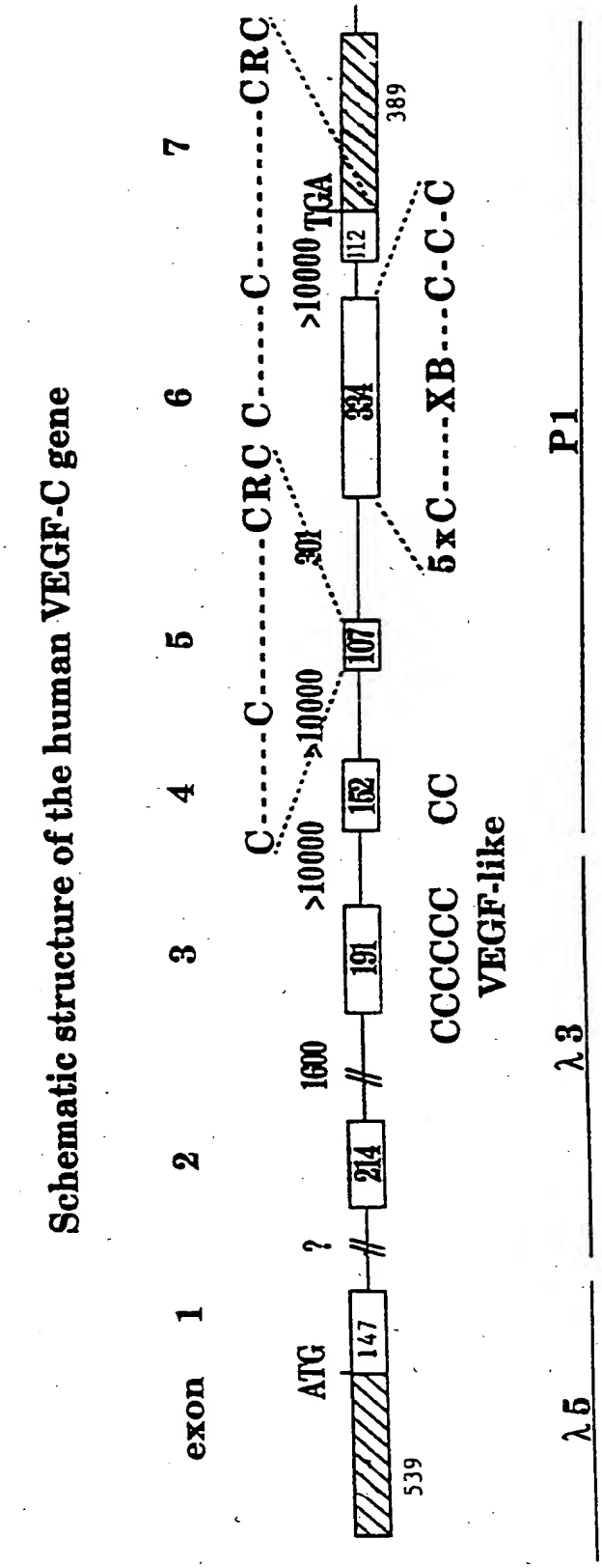
Schematic structure of the human VEGF-C gene
exon 1 2 3 4 5 6 7
ATG ? 1600 >10000 >10000 301 >10000 TGA
539 147 214 191 162 107 334 112 389
CCCCCC CC
VEGF-like
5xC----XB---C-C-C
C-----C-------CRC C-----C-------CRC
λ5
λ3
P1

FIGURE 12

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 98/01973

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/52 C12N15/10 C07K16/24 C12Q1/68
C12N15/62 G01N33/50 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q A01K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOUKOV V ET AL: "A NOVEL VASCULAR ENDOTHELIAL GROWTH FACTOR, VEGF-C, IS A LIGAND FOR THE FLT4 (VEGFR-3) AND KDR (VEGFR-2) RECEPTOR TYROSINE KINASES" EMBO JOURNAL, vol. 15, no. 2, 1996, pages 290-298, XP002022272 see the whole document ---	1-5,7,8, 10,11, 30-34, 37-39,54
X	LEE, J., ET AL. : "vascular endothelial growth fator-related protein: a ligand and specific activator of the tyrosine kinase receptor flt4" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 93, March 1996, pages 1988-1992, XP002066360 see the whole document ---	1-3,10, 11, 30-34, 37,38,54
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 1998	03.07.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 98/01973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39515 A (HUMAN GENOME SCIENCES INC ;ROSEN CRAIG A (US); HU JING SHAN (US);) 12 December 1996 pages 21,34; examples 2,3,4,5,6	1-3,10, 11, 30-34, 36-38, 40,54
X	LEE,J., ETAL. : "VASCULAR ENDOTHELIAL GROWTH FACTOR RELATED PROTEIN (vrp): A LIGAND AND SPECIFIC ACITVATOR OF THE TYROSINE KINASE RECEPTOR Flt4" EMBL SEQUENCE DATA LIBRARY, 10 January 1996, HEIDELBERG, GERMANY, XP002066361 ACCESSION NO. U4142	55,56
X	JOUKOV,V., ET AL. : "A NOVEL VASCULAR ENDOTHELIAL GROWTH FACTOR VEGF-C IS A LIGAND FOR THE FLT4 (VEGFR-3)AND KDR (VEGFR-2) RECEPTOR TYROSINE KINASES" EMBL SEQUENCE DATA LIBRARY, 1 February 1996, HEIDELBERG, GERMANY, XP002066362 ACCESSION NO. X94216	55,56
A	COHEN T ET AL: "VEGF121, A VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ISOFORM LACKING HEPARIN BINDING ABILITY, REQUIRES CELL-SURFACE HEPARAN SULFATES FOR EFFICIENT BINDING TO THE VEGF RECEPTORS OF HUMAN MELANOMA CELLS" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 19, 12 May 1995, pages 11322-11326, XP002061896 see the whole document	1-57
P,X	WO 97 05250 A (UNIV HELSINKI LICENSING ;ALITALO KARI (FI); JOUKOV VLADIMIR (FI)) 13 February 1997 cited in the application pages 10,13-17, 27; examples 8,9,11,14,15,19,22,27,28,29; claims	1-11, 30-34, 36-40, 43,53-56
P,X	JOUKOV,V., ET AL. : "PROTEOLYTIC PROCESSING REGULATES RECEPTOR SPECIFICITY AND ACTIVITY OF VEGF-C" THE EMBO JOURNAL, vol. 16, no. 13, June 1997, pages 3898-3911, XP002066363 see the whole document	1-5, 7-11,14, 15,20, 21, 30-34,54
3 P,X	WO 97 09427 A (GENENTECH INC) 13 March 1997 pages 3,5,9,10,11,24,25,34; example 5,7	1-3,10, 11, 30-38,54
3	-/--	

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INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 98/01973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 97 17442 A (IMMUNEX CORP) 15 May 1997 pages 3,7,8,17,19,21,24; examples	1-3,10, 11, 30-34, 36-38, 40,54
P,X	ACHEN, M.G., ET AL.: "VASCULAR ENDOTHELIAL GROWTH FACTOR D (VEGF-D) IS A LIGAND FOR THE TYROSINE KINASES VEGF RECEPTOR 2 (Flk1) AND vegf RECEPTOR 3 (Flt4)" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 95, January 1998, pages 548-553, XP002066364 abstract, pages 548, right column, page 549, 551; figures 1 + 4;	1-3, 30-34
P,X	JELTSCH,M., ET AL. : "HYERPLASIA OF LYMPHATIC VESSELS IN VEGF-C TRANSGENIC MICE" SCIENCE, vol. 276, 30 May 1997, pages 1423-1425, XP002066365 see the whole document	1-3,10, 11, 30-32, 37-40, 53,54
T	JOUKOV,V., ET AL.: "A RECOMBINANT MUTANT VASCULAR ENDOTHELIAL GROWTH FACTOR-C THAT HAS LOST VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 BINDING , ACTIVATION, AND VASCULAR PERMEABILITY ACTIVITIES" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 12, 20 March 1998, pages 6599-6602, XP002066366 see the whole document	1-57

INTERNATIONAL SEARCH REPORT

Inte...ational application No.
PCT/US 98/01973

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 37-47 and 49,50,52,53 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

The polypeptide analog of claim 13 was searched as refering to the analog mentioned in claim 12.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ·al Application No
PCT/US 98/01973

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639515 A	12-12-1996	AU 6046796 A	24-12-1996
		EP 0837934 A	29-04-1998
WO 9705250 A	13-02-1997	AU 6616996 A	26-02-1997
		EP 0842273 A	20-05-1998
WO 9709427 A	13-03-1997	AU 7012896 A	27-03-1997
WO 9717442 A	15-05-1997	AU 1116297 A	29-05-1997